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Diet-induced thermogenesis in a carnivore, the arctic fox, *Alopex lagopus*

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University of Alaska Fairbanks, 1986

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DIET-INDUCED THERMOGENESIS IN A CARNIVORE, THE ARCTIC FOX, *ALOPEX LAGOPUS*

A

THESIS

Presented to the Faculty of the University of Alaska

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

By

Peter George Tallas, B.S., M.S.

Fairbanks, Alaska

December 1986

DIET-INDUCED THERMOGENESIS IN A CARNIVORE,

THE ARCTIC FOX, ALOPEX LAGOPUS

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ABSTRACT

Carnivores consume diets low in carbohydrate and high in protein and fat. The high dietary levels of protein and fat are thought to contribute greatly to diet-induced thermogenesis (DIT), i.e. the increase in metabolic rate associated with feeding. Low dietary levels of carbohydrate cause the carnivore to stress gluconeogenesis. Consequently, a well developed capacity for gluconeogenesis may be an important adaptation in the carnivorous arctic fox (Alopex lagopus), and may participate in DIT.

The objectives of this study of the arctic fox were to (i) determine DIT associated with four diets that varied in the proportion of fat, protein, and carbohydrate, and (ii) assess glucose utilization in the fed and fasted arctic fox. Fox were fed four diets, (high protein, high fat, high carbohydrate, and high protein/fat) at three levels of energy intake (sub-maintenance, near/above maintenance, and above maintenance). Pre- and postfeeding metabolic rate were measured by open circuit indirect calorimetry. The results indicate that (i) DIT contributes significantly to total heat production of the fox, but is dependent on diet type and energy intake, (ii) DIT is non-existent at sub-maintenance energy intake, regardless of dietary

nutrients, and (iii) the high fat diet is associated with the highest prefeeding and postfeeding metabolic rate at sub-maintenance energy intake, although DIT is non-existent at all levels of energy intake.

For the assessment of glucose turnover, four arctic fox were fed, over a long term, a low carbohydrate, high protein/fat diet. Fed and fasted fox were injected intravenously with radiolabeled glucose, and their blood assayed over time for disappearance of the labeled glucose. The results indicate that glucose metabolism, i.e. total entry rate and irreversible loss, is high compared to other animals, and may support the high blood glucose concentrations of the arctic fox, but does not participate in DIT.

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GENERAL INTRODUCTION

Little research has been conducted on the metabolism of carnivores. The carnivorous arctic fox normally consumes the flesh of other animals, and consequently its diet is high in protein and fat, but negligible in carbohydrate. However, glucose is the primary source of energy for several tissues, such as the brain, nerves, erythrocytes, kidney medulla and testis. Therefore, to satisfy the continual requirement of these tissues for glucose, the carnivorous arctic fox must stress certain metabolic reactions, such as gluconeogenesis, the de novo synthesis of glucose from non-carbohydrate sources (protein and triglycerides). Consequently, a well developed capacity for, and control of, gluconeogenesis may be important adaptations in carnivores.

The high amounts of protein and fat in the carnivorous diet not only may cause the animal to stress gluconeogenesis, but also are thought to contribute greatly to the phenomenon of diet-induced thermogenesis, the increase in metabolic rate associated with feeding. Some have suggested that glucose metabolism may be

involved in this thermogenic phenomenon, specifically, the recycling of glucose carbon fragments, such as lactate, alanine, and glycerol, back into the glucose molecule.

The objectives of the experiments in the arctic fox were first, to determine the diet-induced thermogenesis associated with four diets that varied in the proportion of the major nutrients: fat, protein, and carbohydrate, second, to characterize the association of those individual nutrients with diet-induced thermogenesis, and third, to assess glucose utilization in fed and fasted arctic fox, maintained on a diet similar in composition to the food naturally available in the wild. The specific hypotheses related to these objectives are outlined in the introduction to each individual chapter.

Chapter 1

DIET-INDUCED THERMOGENESIS

1.1 Introduction

Depending on the availability of prey, or on food preference, northern predators have a varied and possibly seasonal dietary ratio of fat to protein. An abundance of various food items offers the predator a choice of a high fat diet, e.g. eggs or blubber, a high carbohydrate diet, e.g. berries, or a high protein diet, e.g. microtine rodents or hare (Lepus spp). Some predators have definite food preferences. For example, winter observations indicate that the polar bear (Ursus maritimus) prefers a high fat diet, as it feeds mostly on the blubber of its prey, the seal, and only consumes the meat last, if at all (Novikov, 1962; Stirling and McEwan, 1975). Conversely, depending on the locale and season, a limited availability of prey may restrict predators at a fixed and non-preferred ratio of nutrients.

Little is known about the metabolic implications of these dietary nutrient ratios. What is known is that the nutrients which are high in a carnivore's diet, protein and fat, are involved in the phenomenon called diet-induced thermogenesis (DIT) (Rothwell and Stock, 1983a). DIT refers to the increase in metabolic rate associated not only with the ingestion of a single meal, but also with the plane of nutrition, i.e. the general level of energy intake.

The metabolic rate of an animal, within its thermoneutral range, is thought to be a function of heat produced due to the inefficient use of chemical energy as the animal carries out functions required for life. These requirements include those of maintenance, activity, and production. Energy for maintenance is used not only for mechanical movements, such as of the heart and respiratory muscles, but also for the molecular syntheses that support cell turnover and the ionic pumps that maintain ionic gradients. The net energy available for the needs of the animal is the energy consumed less the energy of fecal, gaseous (e.g. methane), and urinary losses, and less the energy represented by DIT.

The goal of this study was to characterize DIT in a highly adapted arctic carnivore. The arctic fox (Alopex

lagopus) was chosen and investigated to determine the extent to which DIT may have adaptive significance.

Agriculturalists have long been interested in the relation of DIT to energy balance, since understanding the control of this "waste" heat has special significance for the economics of animal production, such as meat and milk (Maynard et al., 1979; NRC, 1976, 1977, 1978b). Recently, research directed at explaining this thermogenic response of feeding has accelerated, mostly due to increased interest in the cause and treatment of human obesity. Obesity may be a result of a diminution of the thermic effect of food metabolism, rather than simple gluttony (Webster, 1983; Trayhurn and James, 1983).

The increase in metabolic rate caused by feeding was recognized in the late eighteenth century by the French nutritionist Lavoisier (Kleiber, 1975). Over the years, this increase in metabolic rate, induced by feeding, has accumulated a host of synonymous and related terms, such as specific dynamic effect (SDE) (Rubner, 1902), specific dynamic action (SDA) (a translation error from German to English of the previous term), luxuskonsumption (Neumann, 1902), heat increment (Rubner, 1902), thermic effect of feeding (Schwartz et al., 1985), thermic response of food (Nair et al., 1983), and most recently, nutrient-induced thermogenesis (Vernet et al., 1986).

This plethora of names is due partially to the fact that two schools of nutritionists historically have investigated the phenomenon: the ruminant school and the school concerned primarily with nonruminants. The ruminant nutritionists recognized early that metabolic heat production of an animal, at rest and in thermoneutrality, is a direct function of its metabolizable energy intake (Marston, 1948). They called this incremental increase in heat production the heat increment of feeding (HIF) (Webster, 1983). Those investigators working with nonruminants, such as dogs, employed short term trials of a few hours, in contrast to the long term trials, over 24 hours, employed by the ruminant nutritionists. From these studies Rubner (1902) coined the term specific dynamic effect, for the increase in metabolic rate that was apparent after the dog ate its meal (Kleiber, 1975). The short term aspect of SDE has been the basis of criticism from the ruminant nutritionists. Some feel that the short trial period does not allow a complete analysis of the effect of feeding on metabolic rate (Blaxter, 1962). In theory, HIF and SDE are synonymous, since they both refer to the incremental

increase in heat production associated with an increase in metabolizable energy intake (Webster, 1983).

DIT is the term used increasingly today to describe both the short and long term increases in metabolic rate associated with feeding. It is believed to consist of two components: obligatory and regulatory DIT. The former refers to the energy costs of chewing, digesting, absorbing, and processing and/or storing the nutrients in a meal (Trayhurn and James, 1981). Thus, obligatory DIT should depend upon the form and texture of the food and the metabolic fate of nutrients. Regulatory DIT, also described as adaptive or facultative DIT, is that extra heat produced when energy intake is increased far in excess of requirements. Regulatory DIT is thus an incremental increase in metabolic rate which represents a homeostatic mechanism to maintain body weight in the face of increased feeding (Barnes, 1976). However, the increase in metabolic rate resulting from hyperphagia is variable depending on the physiological state of the animal. Hyperphagic, lactating mice exhibit a decrease in thermogenesis, that presumably allows for a greater efficiency for milk synthesis (Trayhurn et al., 1982; Gerardo et al., 1985). Regulatory DIT could also act

adaptively in thermoregulation if environmental conditions warranted it.

Obligatory DIT was originally called SDE by Rubner (1902), who showed that its magnitude was determined by the specific nutrient composition of the meal fed. Rubner (1902) demonstrated that, in the dog, the greatest stimulation of metabolism was produced by a high protein meal, while sugar and fat elicited lesser effects.

Regulatory DIT was originally called luxuskonsumption by Neumann (1902) who noted the constancy of his own body weight despite variations in his energy intake. Subsequent studies in hyperphagic humans have lent support to the conclusions of Neumann (Apfelbaum et al., 1971; Gulick, 1922; Miller and Mumford, 1967; Miller et al., 1967; Sims et al., 1973). More recently, regulatory DIT has been described in rats that were persuaded to overeat a highly palatable diet, e.g. cookies, cold cuts, chips, nuts, etc., usually reserved for humans (Rothwell and Stock, 1979). The feeding of this "cafeteria" diet to rats was thought to involve changes in the activity of brown adipose tissue (BAT) and the sympathetic nervous system (Rothwell and Stock, 1983a). These conclusions were not without criticism by investigators that questioned the validity of the experiments (Armitage et

al., 1981a, 1981b; Barr and McCracken, 1982, 1983; Hervey and Tobin, 1983).

Since the early experiments of Rubner (1902), investigations of the effect of nutrient quality and quantity upon DIT have produced such equivocal results that DIT's mechanism and control remain to be completely elucidated. Recent studies of humans have confirmed that the thermic effect of protein is greater than that of an isoenergetic meal of fat or carbohydrate (Karst et al., 1984; Nair et al., 1983; Welle et al., 1981]). However, others (Garrow, 1973; Pittet et al., 1974) have challenged the concept of the nutrient specificity of a meal's thermic effect and its dependency on energy density. Pittet et al. (1974) suggest that the larger SDA for protein in early experiments with dogs was due to the excitement over the prospect of eating a large and savory meat meal. Subsequent work has shown that the palatability of a meal can significantly contribute to the immediate metabolic effect of that meal (Diamond et al., 1985; LeBlanc and Diamond, 1986; LeBlanc and Brondel, 1985). Nevertheless, most studies support the nutrient specificity hypothesis (Jequier, 1983).

The increase in metabolic rate following ingestion of protein was initially ascribed to the heat produced during amino acid degradation and the energy required for urea

synthesis (Krebs, 1964). While there is partial support for Kreb's original hypothesis (Dauncey and Bingham, 1983), generally there is lack of evidence to corroborate this theory (Garrow and Hawes, 1972), leading others to speculate that the increase in metabolism after a protein meal is more likely caused by an increase in body protein turnover (Brooke and Ashworth, 1972; Grisolia and Kennedy, 1966; Miller et al., 1979).

The protein content of a diet is also implicated as affecting regulatory DIT. Pigs that were fed, ad libitum, a low protein diet (2.6% crude protein dry matter (dm) basis) consumed 5 times as much energy as those pigs fed a high protein diet (26%), yet both groups maintained a constant body weight (Miller and Payne, 1962). However, the results are confounded since changes in body composition could account for the disposition of energy to DIT and body retention (Blaxter, 1973; McCracken and McAllister, 1984). A subsequent study with weanling pigs supported the conclusion that low dietary protein content can affect metabolic rate, i.e. by inducing regulatory DIT (Gurr et al., 1980), leading Rothwell and Stock (1981b) to suggest that this may represent a mechanism for garnering more nutrients from a marginal diet, while disposing of the excess energy as heat, presumably by the sympathetic activation of brown adipose tissue. This hypothesis,

which is supported by Swick and Gribskov's (1983) work in the rat, leads to speculation that wild carnivores forced to eat food of low nutrient (protein) content may also show regulatory DIT. However, before this can be seriously suggested, the effect of carbohydrate and fat on metabolism must be considered.

Depending upon the experiment, carbohydrates have been demonstrated to have a variable effect on DIT. When pigs were fed a high-protein supplement, they reacted classically with an elevation in metabolic rate, but a carbohydrate supplement tended to lessen the metabolic rate below the control level (Dauncey and Ingram, 1979). Subsequent studies in piglets confirmed the minimal aspects of carbohydrate induced thermogenesis (Ingram and Dauncey, 1984). These studies contrast to those with humans, in which an equal thermic effect was associated with both a fat or glucose meal (Welle et al., 1981). A partial explanation of these between-species differences may again be related to nutrient specificity as the metabolic response after a meal of carbohydrate depends upon the particular sugar ingested (Macdonald, 1984; Macdonald and Russell, 1983; Sharief and Macdonald, 1980), and the nutrient composition of the antecedent diet (Acheson et al., 1984).

Evidence for a specific effect of dietary fat on metabolic rate is equally uncertain. Originally, Rubner (1902) found little difference between the SDE's of carbohydrate and fat, with carbohydrate offering the least effect. Later studies on lean and obese subjects indicated that the thermic effect of feeding a high carbohydrate meal was greater than that of a high fat meal (Schwartz et al., 1985). Karst et al. (1984), on the other hand, found no evident thermic response for high fat meals based on butter or sunflower oil. Kasper et al. (1973) fed diets of increasing fat content to normal weight and obese subjects, and observed slight weight gains inconsistent with energy intake. The normal weight subjects, at intakes of fat greater that 300 g/d, reported a marked increase in the bodily sensation of heat and in the tendency for sweating.

Although not supported by the data of Schwartz et al. (1985), others (e.g. Zed and James, 1982) suggest that the thermic responses associated with diet specificity in man may be dependent upon body composition of the subjects. For example, when the diets of lean subjects were supplemented with fat, the resultant thermic response was twice that of obese subjects.

Temperature acclimation can also affect the response elicited by nutrients. For warm-acclimated rats,

carbohydrate feeding produced a slightly greater response in metabolic rate than fat feeding, while the situation was reversed for the cold-acclimated animals (Rothwell and Stock, 1983b). The thermogenic activity of BAT of cold-acclimated mice and rats has been increased by fat feeding (Mercer and Trayhurn, 1984a, 1984b).

Similar increases in thermogenic capacity were found in Siberian hamsters (Phodopus sungorus sungorus) after long-term, high fat feeding (McElroy et al., 1986). Syrian hamsters (Mesocricetus auratus) also exhibited activation of BAT thermogenesis when chronically fed a high fat diet (Hamilton et al., 1986). High fat diets have also been shown, in the genetically obese mouse (ob/ob), to engender hypertrophy of BAT, and to increase its sympathetic nervous activity and overall thermogenic response. This increases cold tolerance in this species, and prolongs its survival in the cold (Himms-Hagen et al., 1986).

The marked effect of diet on metabolic rate has led several to propose that a partial replacement of cold-induced thermogenesis by DIT may be possible (Dauncey, 1979, 1981; Kuroshima et al., 1976, 1977; Leblanc, 1957; Stirling and Stock, 1968; White, 1979; Yuragi and Yoshimura, 1975; Yoshimura et al., 1972). Indeed, Lusk (1933) ends his brief history of nutrition by reflecting:

> "Rubner defined this action as 'specific dynamic action' of the various foodstuffs. These values can be determined only if all the experiments are made when the body is free from the stimulating effect of cold, which itself induces an increase in the heat production. If one enters a restaurant on a cold winter day with a keen sense of chill and one there partakes of a meal of meat, bread, butter, and potatoes, the fires in the body are increased and, on returning to the bitter cold of the outside air, one feels it no longer. The heat of the specific dynamic action of the foodstuffs replaces the disagreeable subjective sensation of exposing the mechanism of chemical regulation to cold. the suffering from cold is intensified by suffering from lack of food."

Little research has been conducted on DIT in wild carnivores. That work accomplished has utilized natural prey as the food source. The species utilized in those studies include: Bering Sea harbor (Phoca vitulina richardsi) and spotted seal (Phoca largha) (Ashwell-Erickson and Elsner, 1981), sea otter (Enhydra lutris) (Costa and Kooyman, 1984), harp seal (Phoca groenlandica) (Gallivan and Ronald, 1981), kestrel (Falco tinnunculus) (Kirkwood, 1981), and largemouth bass (Micropterus salmoides) (Beamish, 1974).

The high proportions of protein and fat in the diets of carnivores are thought to have selected for metabolic adaptations because, in felids, specific dietary requirements, such as for the amino acids arginine and taurine, have evolved (Morris, 1984). The polar bear's preference for fat over protein (Stirling and McEwan,

1975) may be an expression of a metabolic adaptation to the severe conditions of the high arctic, such as aridity and extreme cold. The requirement for dietary water would be decreased, first, by the low protein diet, as there would be fewer products of protein metabolism, e.g. urea, to excrete, and second, by the significant quantities of metabolic water produced by oxidation of fat.

However, none of the carnivore studies have addressed the possibility that DIT may be modified by natural selection to decouple regulatory DIT from obligatory DIT for either increased nutrient acquisition or for sparing thermoregulatory heat production. This study examines the energy metabolism of the carnivorous arctic fox in relation to its dietary nutrient ratio of protein, fat, and carbohydrate, and its influence on DIT.

As a carnivore, the arctic fox feeds on prey items that vary seasonally and regionally. During the summer, major prey include collared (Dicrostonyx torquatus) and brown lemmings (Lemmus sibiricus), which are high in protein and low in fat, and birds and eggs, which are higher in fat. Fish, caribou (Rangifer tarandus), muskoxen (Ovibus muschatus), seamammals and insects are also fed upon, but to a lesser extent. In the summer, the relative protein and fat content of these prey are highly variable. The large mammals would, of course, be eaten as

carrion. Berries and grasses are sometimes taken, but never in quantities that would classify the arctic fox as an omnivore. During the winter, the diet of fox on sea ice consists of scavenged marine mammal carcasses and resident sea birds. Those fox remaining inland on the tundra eat mainly lemmings, caribou carcasses, hare and ptarmigan (Lagopus spp.) (Chesemore, 1968; Garrot et al., 1983; Riewe, 1977; Speller, 1972). Consequently, the fox's natural diet is high in those components that are thought to contribute greatly to DIT, i.e. protein and fat. Studies of food habits and basic metabolic investigations may reveal whether the arctic fox has adapted DIT for survival in the severe arctic environment.

This study tests the hypotheses that:

1. DIT contributes significantly to the total heat production of the arctic fox.
2. DIT is adaptive if it is highest for those nutrients that comprise a carnivorous diet, protein and fat.
3. DIT is generally directly related to the energy level of the diet.
    a. At low levels of energy intake (starvation), DIT will be low, because a "wasting" of ingested energy would be non-adaptive.

b. At higher levels of energy intake, DIT will level off, as it would be more advantageous not to "waste" too much of the ingested energy as heat, so as to allow for more fat deposition.

The objectives of these experiments in the arctic fox were, first, to determine the DIT associated with four diets that varied in the proportion of the major nutrients: fat, protein, and carbohydrate, and second, to characterize the association of those individual nutrients with DIT.

1.2 Materials and Methods

1.2.1 Animals

The four littermate arctic fox used in this study were seven years old and weighed approximately 3-4 kg. They were obtained from the Naval Arctic Research Laboratory at Barrow, Alaska. Fox were housed separately in concrete floored pens (224 cm · 112 cm · 345 cm), whose wire-mesh upper portions were open to the outside, allowing the area to be maintained at the seasonal ambient temperature and light. Each animal had a shelter box and water available at all times. The experimental period included the months of February through May 1984.

1.2.2 Diets

When not participating in experiments the fox were fed solely a commercially canned diet specially prepared for non-domesticated carnivores (Zupreem Feline, Hill's Animal Care Products, Topeka, Kansas) and had water available at all times.

1.2.2.1 --Digestibility of Zupreem--

The apparent dry matter digestibility (ADMD) of 3 levels of intake of Zupreem was determined in June, July, and August 1983 using the non-digestible marker, ^{51}Cr complexed with ethylenediamine tetraacetic acid (^{51}Cr) (New England Nuclear, Boston, Massachusetts).

The digestibility of 60 g dm/d of Zupreem was determined first. After 4 weeks of ad libitum feeding, consecutive feeding trials were performed to assess the digestibility of Zupreem at 120 g dm/d and 30 g dm/d.

The Zupreem was labeled with an appropriate aliquot of ^{51}Cr and fed to the fox once daily for a period of 5 to 6 days. The feeding regime was continued for the next 3 to 4 days with Zupreem but without the isotope. Fecal samples were collected daily from each fox over the entire 8 to 10 days. The feed and fecal samples were put into vials, freeze-dried to a constant weight and then assayed for their ^{51}Cr content (Searle Analytic Gamma System 1195).

Per cent dry matter digestibility was calculated by comparing the gamma radiation emitted from the labeled dried food samples with that from the dried fecal samples according to the equation:

$$\frac{^{51}Cr_{feces} - {}^{51}Cr_{food}}{^{51}Cr_{feces}} \cdot 100 = \% \text{ dry matter digestibility}$$

where $^{51}Cr_{feces}$ is the isotope activity in counts per min (cpm) per g of dry feces, and $^{51}Cr_{food}$ is the activity in cpm per g of labeled dry food (Robbins, 1983). A mean value of digestibility was calculated from the individual

ADMD values of the last 3 fecal samples before the ^{51}Cr was removed from the diet.

For determination of the turnover time (TT) of food in the gastrointestinal tract, i.e. the time required for the amount of food equal to the volume of the tract to move through the tract, a least-squares regression line was fitted to the natural logarithm of the ^{51}Cr activity per g of dry feces vs time (d) for the 3 d after termination of feeding the labeled Zupreem (Dixon et al., 1981; Holleman et al., 1984). Turnover time was calculated from the regression according to the equation:

$$TT = 1/m$$

where m is the slope of the regression equation:

$$\ln \{SF\ ^{51}Cr\} = \ln \{IF\ ^{51}Cr\} \cdot e^{-mt}$$

where SF is the sample fecal act'ivity in cpm, IF is the initial fecal activity in cpm, and t is the time in h.

1.2.2.2 --Supplemented diets--

Each of the other diets used in the experiments, high fat, high protein, and high carbohydrate, were prepared by supplementing Zupreem (ZUP) with lard (Armour & Co., Phoenix, Arizona), casein (Purified high nitrogen, Nutritional Biochemicals, Cleveland, Ohio) or glucose (Analytic Reagent, Mallinckrodt Inc., Paris, Kentucky) respectively (Table 1). The high fat diet (FAT) was

prepared as 57% Zupreem and 43% lard while the high protein (PRO) and high carbohydrate (CHO) diets contained equal percentages of Zupreem and either casein, 23%, or glucose, 46%.

The protein content of the diets was estimated from the Kjeldahl total nitrogen content. Samples were digested with sulfuric-selenous acid, then analyzed colorimetrically with a Technicon autoanalyzer (laboratory of F.S. Chapin, University of Alaska, Fairbanks). The other proximate constituents, fat, carbohydrate, and water were estimated from the ratios of ingredients used in the preparation of each diet. The manufacturer's analysis of Zupreem indicated it to be 16% fat, 3% carbohydrate, and 60% water (Table 2).

The estimated metabolizable energy (ME) in the diet was calculated using the values of 17, 38, and 17 kj ME/g of carbohydrate, fat, and protein, respectively (NRC, 1982). The four diets differed markedly from one another in percentage contribution of each food component to the total estimated metabolizable energy content of the diet (Table 3).

The protein-energy contribution was highest in the PRO diet, 2.3 times that offered in the ZUP diet, and 5 to 9 times that of the CHO and FAT diets (Table 3). The fat-energy contribution was highest in the FAT diet, 4.3 times

Table 1. Diet composition as a percentage of preparatory ingredients, expressed on a fresh weight basis.

	Diet Type			
	ZUP	FAT	PRO	CHO
Ingredient				
Zupreem	100	57	23	46
Lard	0	43	0	0
Casein	0	0	23	3
Glucose	0	0	0	46
Water	0	0	54	5

Table 2. Nutrient composition (g/100 g dm) and metabolizable energy content (kj/g dm) of Zupreem and supplemented diets.

	Diet Type			
	ZUP	FAT	PRO	CHO
Nutrient		g/100 g dm		
Protein	45	15	79	14
Fat	40	79	12	11
Carbohydrate	8	3	3	73
		kj/g dm		
Energy	24	33	18	18

Table 3. Percent contribution of protein, fat, and carbohydrate to total energy content of diets.

	Diet Type			
	ZUP	FAT	PRO	CHO
Nutrient				
Protein	32	8	72	14
Fat	63	90	25	21
Carbohydrate	5	2	3	65

the value of the CHO diet, 3.6 times that of the PRO diet, but only 1.4 times the percentage offered by ZUP. The CHO diet offered the largest contribution of energy from carbohydrate, 13 times that of the ZUP diet, and 22 to 32 times that of the PRO and FAT diets.

Each diet was fed at three different planes of nutrition over three consecutive 8 d experimental periods. The four diets were randomly assigned to a sequence of feeding (Petersen, 1985). FAT was fed for the first 24 d, followed by ZUP, PRO, and CHO. The low plane (LP) was fed at 450 kj/d, the near maintenance or medium plane (MP) at 1800 kj/d, and the high plane (HP) at 2700 kj/d ME. Within each diet the sequence of plane feeding was LP, MP, and HP. The diets were freshly prepared and fed as one meal between 1600 and 1700 h.

1.2.3 Experimental protocol

The metabolic chamber (122 cm · 91 cm · 109 cm) used for the measurement of respiratory gases, oxygen and carbon dioxide, was fabricated from clear plexiglas (Figure 1). This chamber could be lowered over a stainless steel metabolic cage that confined the fox. A small tube (2.5 cm diameter) in the top of the chamber allowed the fox to be fed without opening the chamber. Food was forced through the tube and fell into a pan that allowed the fox to eat all that was offered, with no

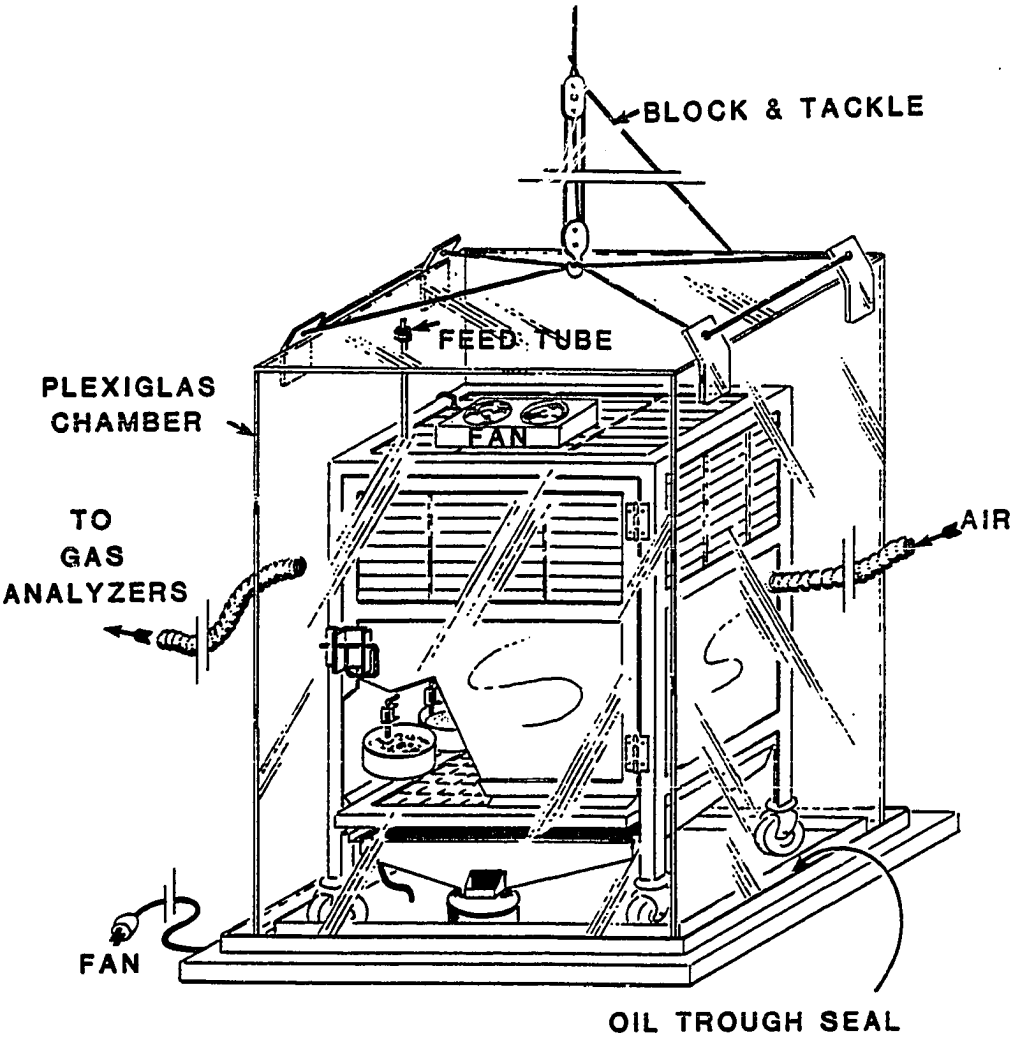

Figure 1. Metabolic cage and chamber

waste. The stainless steel cage was fitted with an open mesh floor, that allowed separation of feces and urine. A sloping pan collected urine into a beaker containing a small amount of HCl for preservation. The plexiglas chamber was lowered over the cage into floor channeling that was filled with mineral oil, effectively sealing the chamber from the outside.

The atmosphere in the chamber was circulated with two 13 cm fans. A 5 cm diameter hose supplied air to the chamber from a rarely-frequented area outside the building. A similar hose exhausted the chamber at a flow rate of 35 liters per minute, which was continuously monitored with a mass flow meter (Model 505-9, Kurz Instruments Inc., Carmel Valley, California).

Temperature and relative humidity of the air flow from the chamber was determined with a hygrometer (Model HT-1, Jersey Technical Electronics, Inc., Middlesex, New Jersey). A subsample from the main flow was withdrawn, dried by passage through a 2.5 cm diameter column of $CaSO_4$ (W.A. Hammond Drierite Co., Xenia, Ohio), and its oxygen content measured with a paramagnetic analyzer (Model F3, Beckman Instruments, Inc., Fullerton, California) and carbon dioxide content determined with an infrared analyzer (Model 864, Beckman Instruments Inc., Fullerton, California). These gas analyzers were calibrated with gas

mixtures assessed with the Scholander 0.5cc gas analyzer (Holker Scientific Instruments, Swarthmore, Pennsylvania). The gas analyzers, barometer (Model 1520, Sierra-Misco, Berkeley, California), temperature sensor, and flow meters were interfaced to a minicomputer (Eclipse S-140, Data General, Westboro, Massachusetts) for controlled data acquisition (Kokjer, 1981).

At approximately 0900 h on the first morning of an experiment, on the eighth day of the feeding regime, a fox was removed from its outdoor cage via a transportation cage (76 cm · 30 cm · 30 cm). After the fox was weighed, it was put into the metabolic cage, over which the plexiglass chamber was lowered and sealed. Water was available ad libitum. Although the pump that drew outside air through the chamber was begun immediately before the fox was put into the chamber, data collection was not initiated for 2 h (at approximately 1200 h) to allow equilibration of the chamber atmosphere. During this procedure, the fox was regularly relaxed, assuming a lying and resting posture soon after the chamber was closed. Feeding, with freshly prepared food, occurred 6 h after the start of data collection. Food consumption was complete within 2 min. The fox usually settled to a resting posture after feeding. Data collection was continued through that night until the end of the

experiment the following day, at approximately 1600 h; a total trial period of 30 h. After the fox was returned to its outside run, urine was removed from the collection receptacle, its pH adjusted for acidity, and stored frozen at -30° C until analysis.

1.2.4 Calculations

Calculation of oxygen consumption began with determination of chamber air out-flow rate corrected to STP (0° C, 760 mm Hg). To calculate gaseous exchange it was necessary to calculate the volumes of air entering the chamber because the input and output differed in composition. Flow rate into the chamber, $Flow_{in}$ (l/min), was calculated with the equation:

$$Flow_{in}=Flow_{out}\cdot\{100-O_{2(out)}-CO_{2(out)}\}\cdot\{1/[(100-O_{2(in)}-CO_{2(in)})]\}$$

Where $Flow_{out}$ (l/min) is the measured flow out of the chamber, $O_{2(out)}$ and $CO_{2(out)}$ are the l of O_2 and CO_2, respectively, per l of gas leaving the chamber, and $O_{2(in)}$ and $CO_{2(in)}$ are the percentages of oxygen and carbon dioxide, respectively, in atmospheric air.

Oxygen consumption ($\dot{V}O_2$, ml/min) of the fox was calculated by the following equation:

$\dot{V}O_2=\{Flow_{in}\cdot O_{2(in)}\}-\{Flow_{out}\cdot O_{2(out)}\}$

Carbon dioxide production ($\dot{V}CO_2$, ml/min) was calculated in a similar manner:

$\dot{V}CO_2=\{Flow_{out}\cdot CO_{2(out)}\}-\{Flow_{in}\cdot CO_{2(in)}\}$

Respiratory quotient (RQ) was calculated as $\dot{V}CO_2/\dot{V}O_2$. These calculations were performed every minute of the experiment from the input of the data acquisition system.

Preprandial resting oxygen consumption is defined as the mean value for the 360 minutes prior to feeding. Postprandial resting oxygen consumption represents the mean value for a continuous 8 hour period beginning 2 hours after feeding. The oxygen consumption due to activity was omitted from the means.

Calculation of weights of carbohydrate, fat, and protein metabolized, energy expenditure, and the metabolic water produced in each trial was made from the urinary nitrogen excretion, total oxygen consumed, and carbon dioxide produced (Consolazio et al., 1963). Liters of CO_2 and O_2 represent the mean of the pre- and postprandial values.

Protein metabolized (g) $=6.25\cdot$(g urinary nitrogen)

Carbohydrate metabolized (g) $= \{-2.54 \cdot (\text{g urinary nitrogen})\} \cdot \{-2.91 \cdot (\text{l } O_2 \text{ consumed})\} \cdot \{4.12 \cdot (\text{l } CO_2 \text{ produced})\}$

Fat metabolized (g) $= \{-1.94 \cdot (\text{g urinary nitrogen})\} \cdot \{1.69 \cdot (\text{l } O_2 \text{ consumed})\} \cdot \{-1.69 \cdot (\text{l } CO_2 \text{ produced})\}$

Metabolic water produced (g) $= \{-1.04 \cdot (\text{g urinary nitrogen})\} \cdot \{0.062 \cdot (\text{l } O_2 \text{ consumed})\} \cdot \{0.662 \cdot (\text{l } CO_2 \text{ produced})\}$

Energy expenditure (Kj) $= \{11.94 \cdot (\text{g urinary nitrogen})\} \cdot \{15.82 \cdot (\text{l } O_2 \text{ consumed})\} \cdot \{4.87 \cdot (\text{l } CO_2 \text{ produced})\}$

1.2.5 Statistical analysis

Two-way analysis of variance was applied to (i) the dry matter digestion of Zupreem data, using intake level and fox as the factors, and (ii) the weight change data, within each plane of nutrition, using diet group and fox as the factors. Three-way analysis of variance was applied to oxygen consumption, respiratory quotient, and metabolic mixture analyses, using diet group, plane of nutrition, and fox as the factors (Jennrich et al., 1981). Comparisons between means, in those data sets that yielded significant F ratios in their analyses of variance ($P<0.05$), were performed with the "Fisher's protected least significant difference" test criterion, where statistical significance was indicated if P values were

less than 0.05 (Steele and Torrie, 1980). Comparisons of the regression equations, both single and multiple, to detect differences in slope and/or intercepts were accomplished by calculating the test statistic F and specifying the level of significance at 0.05 (Dixon et al., 1981; Neter and Wasserman, 1974). Values are presented as mean $\pm$ 1 standard error of the mean (SEM).

1.3 Results

1.3.1 Zupreem digestibility

A typical experiment relating the radioactivity of dry feces to time after the introduction of the ^{51}Cr to the food is presented in Figure 2. The radioactivity of the feces reached a plateau value within 24 h after the initial consumption of the labeled food. When the labeled food was replaced with unlabeled food the radioactivity quickly decreased, so that within 3 d the activity of the feces was close to background.

The average dry matter digestibilities of the 3 levels of intake, 30, 60, and 120 g/d of Zupreem, were not significantly different from one another (Table 4). Consequently all data were combined to yield a value of 84.2%.

The mean gastrointestinal turnover times of the fox fed the higher levels of Zupreem, 60 and 120 g/d, were not significantly different from one another, and consequently were combined to yield a turnover time of 7.8 h. The turnover time of fox fed at the low level (30 g/d) was 11.4 h, significantly greater than that of the high intake groups (Table 5).

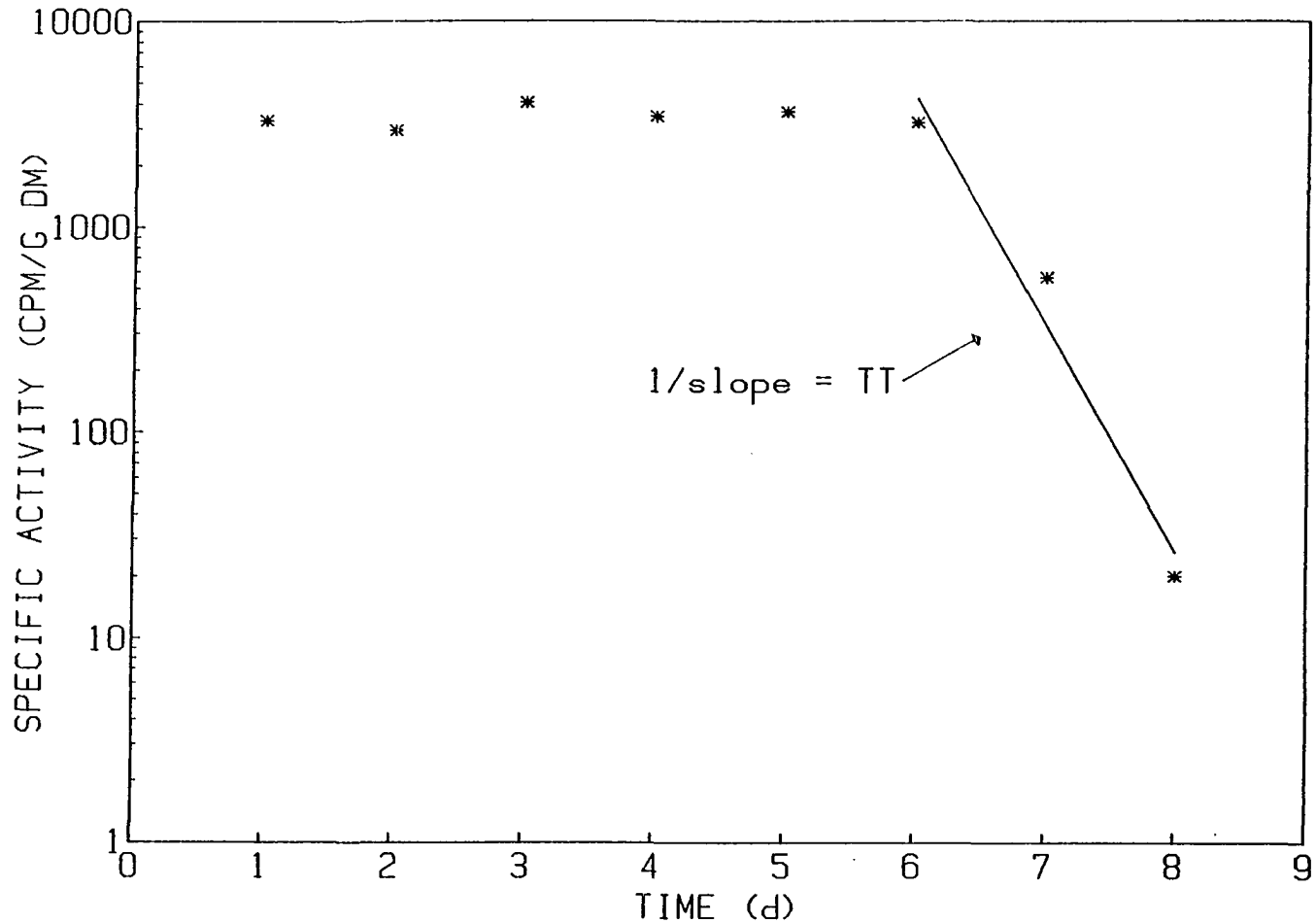

Figure 2. Typical experiment measuring fecal ^{51}Cr excretion in an arctic fox fed ^{51}Cr labeled Zupreem.

Table 4. Percent efficiency of dry matter digestion of Zupreem fed to five arctic fox

	Intake (g dm/d)		
	30	60	120
Fox id			
1	81.2	87.8	84.9
H	80.8	83.7	81.3
2	84.3	87.2	85.1
3	83.7	85.3	84.0
4	83.9	84.3	85.0
Mean±SEM	82.8±0.7	85.7±0.8	84.1±0.7

Means were not significantly different from one another ($P>0.05$), and were combined into one total mean of 84.2±0.5%.

Table 5. Gastrointestinal turnover time (h) in five arctic fox fed Zupreem diet.

	Intake (g dm/d)		
	30	60	120
Fox id			
1	33.7	8.3	6.4
H	12.6	9.2	7.6
2	12.0	7.4	6.7
3	8.6	7.9	6.8
4	7.6	9.4	9.2
All fox	11.4^{a}	8.4^{b}	7.2^{b}

Values not sharing a common letter superscript differ significantly ($P<0.0001$).

1.3.2 Weight changes

Rates of weight change (g/d) of the four fox at all planes of nutrition were not significantly different ($P>0.05$) between diet groups (Figure 3; Table 6).

Linear regression analysis for the relationship between weight change (WC, g/d) and energy intake per fox (EIF, kj/d) of the low and medium planes indicated no significant differences among the 4 diet groups, and the data were fitted to one equation:

$$WC = -94.5 + 0.0638\ EIF \qquad r^2=0.862$$
$$\underset{(0.0047)}{} \qquad P<0.0001 \quad n=32$$

The number in parenthesis below the coefficient represents its standard error. When energy intake was expressed on a metabolic weight basis (EI, $kj/d \cdot kg^{0.75}$), there were no differences between the equations of the diet groups, and the data were fitted to one equation:

$$WC = -90.4 + 0.152\ EI \qquad r^2=0.825$$
$$\underset{(0.013)}{} \qquad P<0.0001 \quad n=32$$

The energy required for maintenance was calculated by setting WC to 0.0 and solving for EIF, 1481 kj/d, or for EI, 595 $kj/d \cdot kg^{0.75}$.

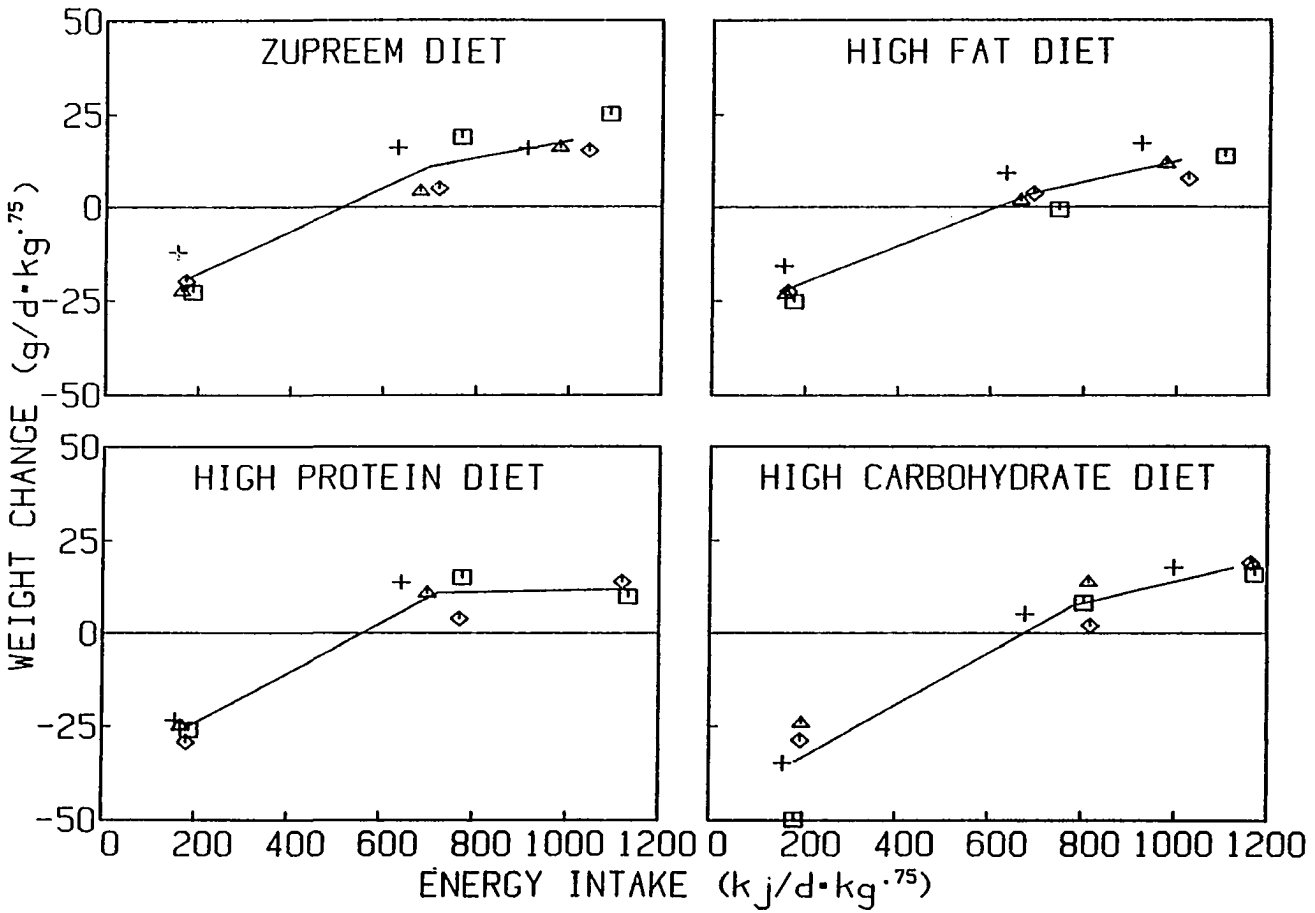

Figure 3. Weight change of four arctic fox in relation to diet and energy intake.

Table 6. Mean weight change (g/d) of four arctic fox in relation to diet and plane of nutrition.

kj/d	ZUP	FAT	PRO	CHO	Significance
450	-51 ±6	-60 ±5	-67 ±2	-86 ±15	NS
1800	28 ±10	10 ±6	27 ±6	17 ±5	NS
2700	48 ±5	34 ±6	28‡ ±24	43 ±3	NS

mean values ± sem, ‡ n=2

1.3.3 Oxygen consumption

1.3.3.1 --Typical trial--

The minute-to-minute pattern of oxygen consumption during a typical experiment is presented in Figure 4. The peak oxygen consumption at 1600 h resulted from the immediate thermic effect of the feeding and its associated activity, while the large peaks rising from the baseline at 2200 and 0200 h were due solely to activity. These activity-induced $\dot{V}O_2$ responses were removed from the analysis, thus assuming a linear relationship between the initial and terminal points of the response curve (Figure 4).

1.3.3.2 --Preprandial oxygen consumption--

Preprandial $\dot{V}O_2$ ($PR\dot{V}O_2$($ml/min \cdot kg^{0.75}$) at the three planes of nutrition (Figure 5; Table 7) was directly related to the fractional contribution of dietary fat (FE), and to a lesser extent protein (PE), to total dietary energy. This relationship is expressed in the following linear regressions (PE was not included in the regression model as its coefficient estimate proved to be insignificant):

$$LP\ PR\dot{V}O_2 = 5.88 + 5.76\ FE \quad (0.84) \qquad r^2=0.772 \quad P<0.0001 \quad n=16$$

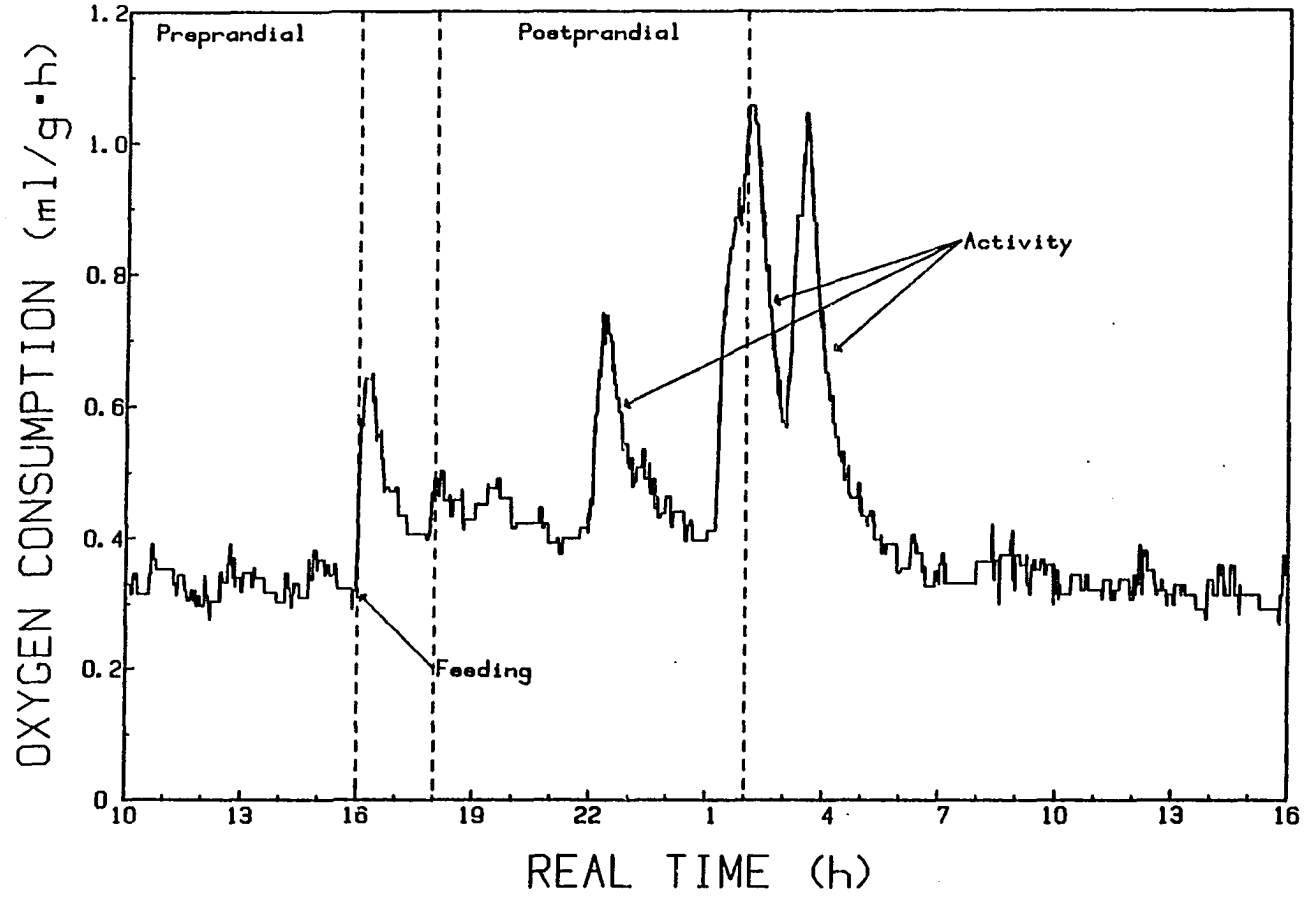

Figure 4. Typical experiment measuring oxygen consumption in an arctic fox fed at near maintenance level. Peaks due to activity at 2200 and 0100 h were omitted from reported means.

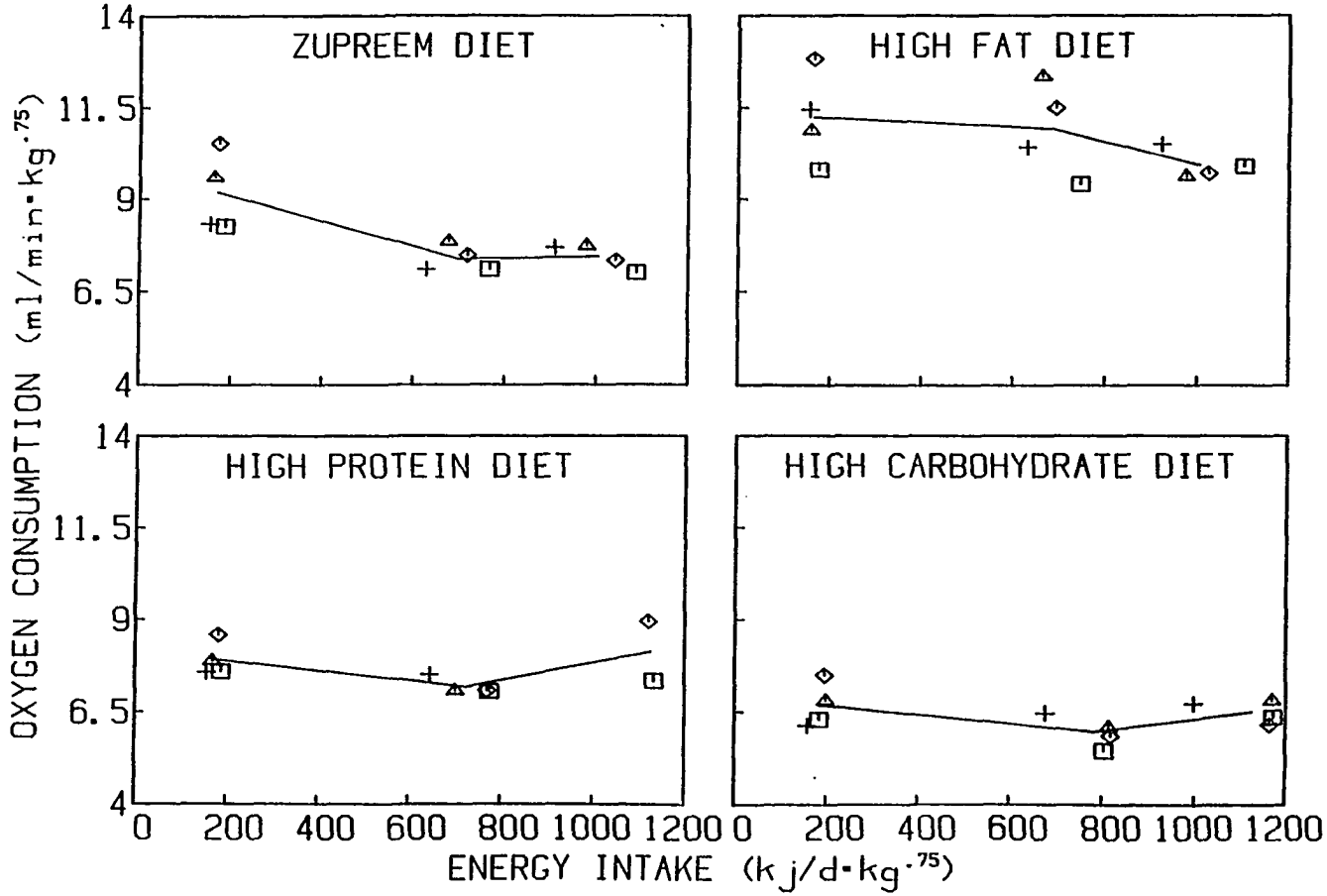

Figure 5. Preprandial oxygen consumption of four arctic fox in relation to diet and plane of nutrition.

Table 7. Preprandial oxygen consumption (ml/min-kg$^{0.75}$) of four arctic fox in relation to diet and plane of nutrition. Values represent the mean oxygen consumption for 6 hours preceeding feeding.

kj/d	ZUP[1]	FAT[2]	PRO[3]	CHO[4]	Significance	
450[A]	9.17 ±0.55	11.24 ±0.63	7.90 ±0.24	6.68 ±0.30	2 vs 1,3,4 1 vs 3,4 3 vs 4	P<0.001 P<0.01,0.001 P<0.01
1800[B]	7.37§ ±0.18	10.90 ±0.64	7.18§ ±0.11	5.96§ ±0.21	2 vs 1,3,4 4 vs 1,3	P<0.001 P<0.005,0.01
2700[C]	7.43§ ±0.17	9.91 ±0.20	8.14‡§ ±0.80	6.53§ ±0.15	2 vs 1,4 2 vs 3 4 vs 1,3	P<0.001 P<0.005 P<0.05,0.01
Significance:	A vs B,C P<0.001	A vs C P<0.01 B vs C P<0.05	NS	NS		

mean values ± sem, ‡ n=2 ; § significantly less than postprandial value (P<0.001).

$$MP\ PR\dot{V}O_2 = 4.97 + 5.79\ FE \qquad r^2=0.722$$
$$(0.96) \qquad P<0.0001 \quad n=16$$

$$HP\ PR\dot{V}O_2 = 5.95 + 3.81\ FE \qquad r^2=0.622$$
$$(0.86) \qquad P<0.0008 \quad n=14$$

The regression equations for MP and HP were not significantly different and were combined into a single equation.

$$MHP\ PR\dot{V}O_2 = 5.42 + 4.85\ FE \qquad r^2=0.664$$
$$(0.65) \qquad P<0.0001 \quad n=30$$

At all planes of nutrition, $\dot{V}O_2$ of the FAT group was significantly greater ($P<0.005$) than that of the other diet groups. In the 2 groups fed the highest concentration of fat, the ZUP and FAT diets, the LP $\dot{V}O_2$ was higher than HP $\dot{V}O_2$. Likewise, in fox fed diets lowest in fat, the CHO diet, preprandial $\dot{V}O_2$ was the lowest of all groups, and no plane of nutrition effect was evident. Intermediate $\dot{V}O_2$'s were noted in the PRO and ZUP groups, but a significant difference between LP and MP $\dot{V}O_2$'s occurred only in the ZUP group. There was no significant difference in the above described relationships and mean trends when $\dot{V}O_2$ was expressed in terms of body weight (ml/g·h) rather than metabolic weight.

1.3.3.3 --Postprandial oxygen consumption--

Postprandial oxygen consumption ($PO\dot{V}O_2$) was directly related to the fat and protein energy content of the diets, as was the case before feeding (Figure 6; Table 8). PE was dropped from the LP regression, while FE was omitted from the HP regression due to the insignificance of the respective coefficient estimates.

LP $PO\dot{V}O_2$ = 6.43 + 4.86 FE (r^2=0.636
(1.02) P<0.0004 n=15

MP $PO\dot{V}O_2$ = 7.40 + 2.59 FE + 4.37 PE (r^2=0.548
(0.98) (1.19) P<0.0085 n=15

HP $PO\dot{V}O_2$ = 9.64 + 2.52 PE (r^2=0.389
(1.00) P<0.0304 n=12

Postprandial $\dot{V}O_2$ at LP followed the same pattern that existed preprandially; i.e. $\dot{V}O_2$ for the FAT diet (11.01±0.87) was significantly higher than that for the CHO group (7.04±0.14), the lowest value of all the groups. $\dot{V}O_2$ of the LP level of the ZUP and PRO groups were intermediate. At MP, the highest $\dot{V}O_2$ was noted for the PRO group (11.44±0.28), while the FAT, ZUP, and CHO diets (8.57±0.50) were associated with progressively lesser values (Table 8). The $\dot{V}O_2$ of the 4 diet groups at HP maintained the same relative order as seen at MP, although only the PRO group was significantly different from the

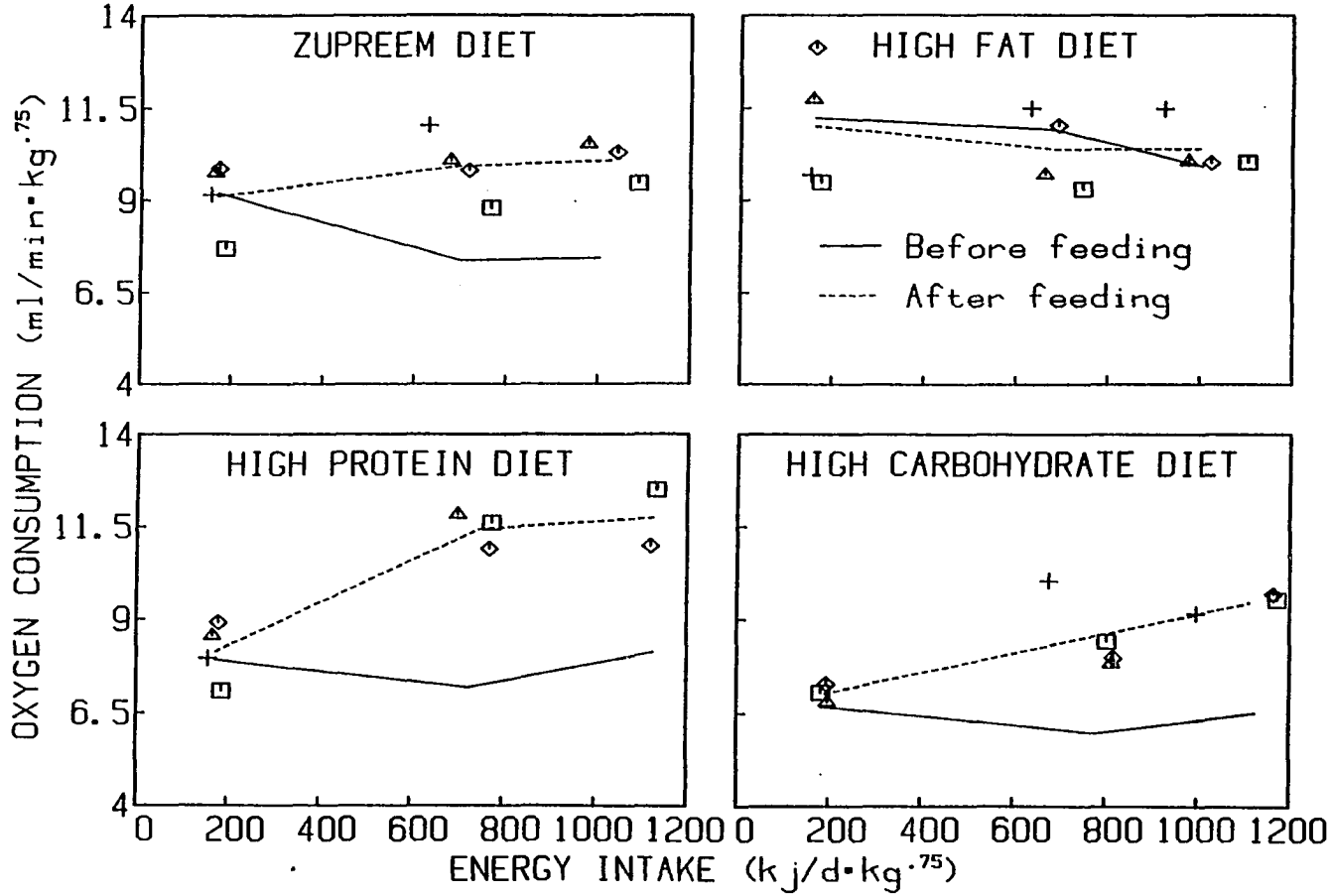

Figure 6. Postprandial oxygen consumption of four arctic fox in relation to diet and plane of nutrition.

Table 8. Postprandial oxygen consumption (ml/min-kg$^{0.75}$) of four arctic fox in relation to diet and plane of nutrition. Values represent the average oxygen consumption for the period between 2 and 10 hours after feeding.

kj/d	ZUP[1]	FAT[2]	PRO[3]	CHO[4]	Significance	
450[A]	9.11 ±0.50	11.01 ±0.87	8.13 ±0.48	7.04† ±0.14	2 vs 1,3,4 1 vs 3,4 3 vs 4	P<0.001 P<0.05,0.001 P<0.05
1800[B]	9.91§ ±0.46	10.37 ±0.53	11.44†§ ±0.28	8.57§ ±0.50	2 vs 3,4 1 vs 3,4 3 vs 4	P<0.05,0.002 P<0.01 P<0.05
2700[C]	10.08†§ ±0.32	10.38 ±0.36	11.74‡§ ±0.76	9.46†§ ±0.16	3 vs 2,1 3 vs 4	P<0.025,0.01 P<0.002
Significance:	NS	NS	A vs B,C P<0.001	A vs B,C P<0.01,0.001		

mean values ± sem, † n=3 ; ‡ n=2 ; § significantly greater than preprandial value (P<0.001).

other 3 groups. The change in energy intake from LP to MP or HP was associated with an increase of $\dot{V}O_2$ only in the PRO and CHO groups. This trend is reverse to that of the preprandial state (Figure 6). The same trends were noted when results were expressed as ml/g·h.

1.3.3.4 --Diet-induced thermogenesis--

Diet-induced thermogenesis (DIT) was calculated as the percentage increase in $\dot{V}O_2$ from the pre- to postprandial condition. The magnitude of DIT was inversely related to the contribution of fat to total energy content of the diet (Figure 7; Table 9). A direct, but weaker relationship existed between protein content and DIT, although its coefficient proved to be insignificant at HP. The regression equations describing these relationships at MP and HP intake are below. The regression was insignificant for the LP diet groups.

MP DIT = 50.8 - 59.4 FE + 41.4 PE r^2=0.791
(13.6) (16.6) P<0.0001 n=15

HP DIT = 63.2 - 59.3 FE r^2=0.664
(13.6) P<0.0015 n=12

Similarly, direct relationships existed between DIT and fat and protein intake (FI and PI, g/d) at MP and HP. Expressed in this manner, the coefficient for protein intake still contributed significantly to this strong relationship at MP.

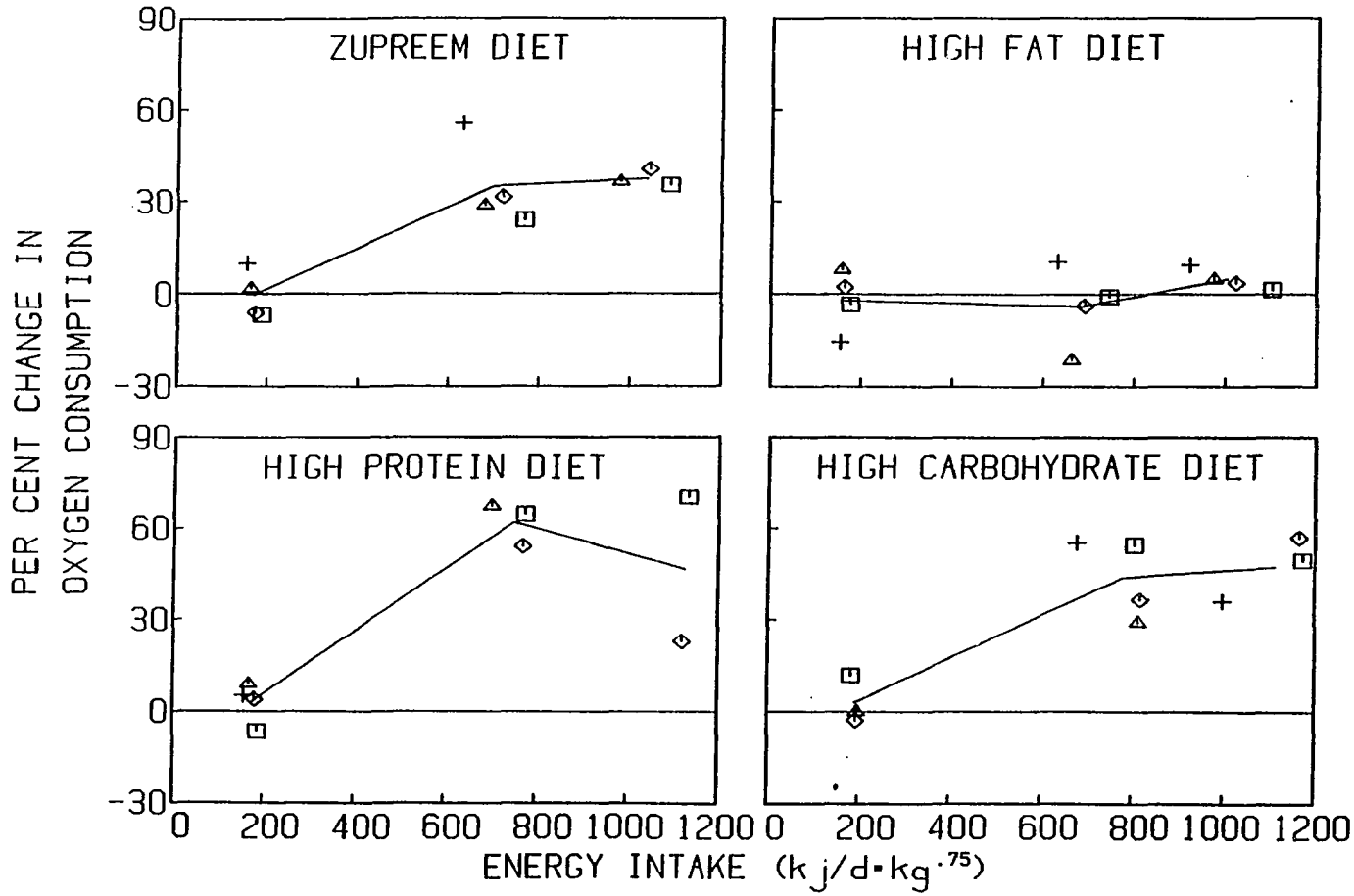

Figure 7. Diet-induced thermogenesis as change from pre- to postprandial oxygen consumption of four arctic fox in relation to diet and plane of nutrition.

Table 9. Diet-induced thermogenesis as per cent change from pre- to postprandial oxygen consumption of four arctic fox in relation to diet and plane of nutrition.

kj/d	ZUP[1]	FAT[2]	PRO[3]	CHO[4]	Significance	
450[A]	-0.4 ±3.9	-2.1 ±5.0	2.9 ±3.3	3.1† ±4.5	NS	
1800[B]	34.6 ±7.1	-4.0 ±6.6	61.9† ±4.0	43.8 ±6.6	2 vs 1,3,4 1 vs 3 3 vs 4	P<0.001 P<0.01 P<0.05
2700[C]	37.3† ±1.7	4.7 ±1.7	46.5‡ ±23.6	47.4† ±6.1	1 vs 2 2 vs 3,4	P<0.002 P<0.001
Significance:	A vs B,C P<0.001	NS	A vs B,C P<0.001	A vs B,C P<0.001		

mean values ± sem, n=4; † n=3 ; ‡ n=2

MP DIT = 50.8 - 1.25 FI + 0.39 PI r^2=0.791
(0.29) (0.16) P<0.0001 n=15

HP DIT = 63.2 - 0.83 FI r^2=0.980
(0.19) P<0.0001 n=12

At MP the highest DIT (61.9±4.0%) was noted for the PRO diet, while the FAT diet was associated with a decrease in $\dot{V}O_2$ (-4.0±6.6%). DIT's in the ZUP and CHO groups were intermediate and not significantly different from one another. The same results were noted when fox were fed at HP. DIT of all the diets except FAT increased significantly from LP to MP, suggesting that DIT is only manifest once energy intake exceeds maintenance, provided the diet has a sufficiently low fat content, or conversely, a high protein/carbohydrate content.

1.3.3.5 --Respiratory quotient--

Preprandial RQ (PRRQ) varied inversely to the fat and protein contribution to dietary energy (Figure 8; Table 10). The PE coefficient was omitted from the following regressions since it was insignificant.

LP PRRQ = 0.920 - 0.086 FE r^2=0.374
(0.030) P<0.0118 n=16

MP PRRQ = 1.05 - 0.237 FE r^2=0.628
(0.049) P<0.0003 n=16

HP PRRQ = 1.04 - 0.217 FE r^2=0.494
(0.063) P<0.0051 n=14

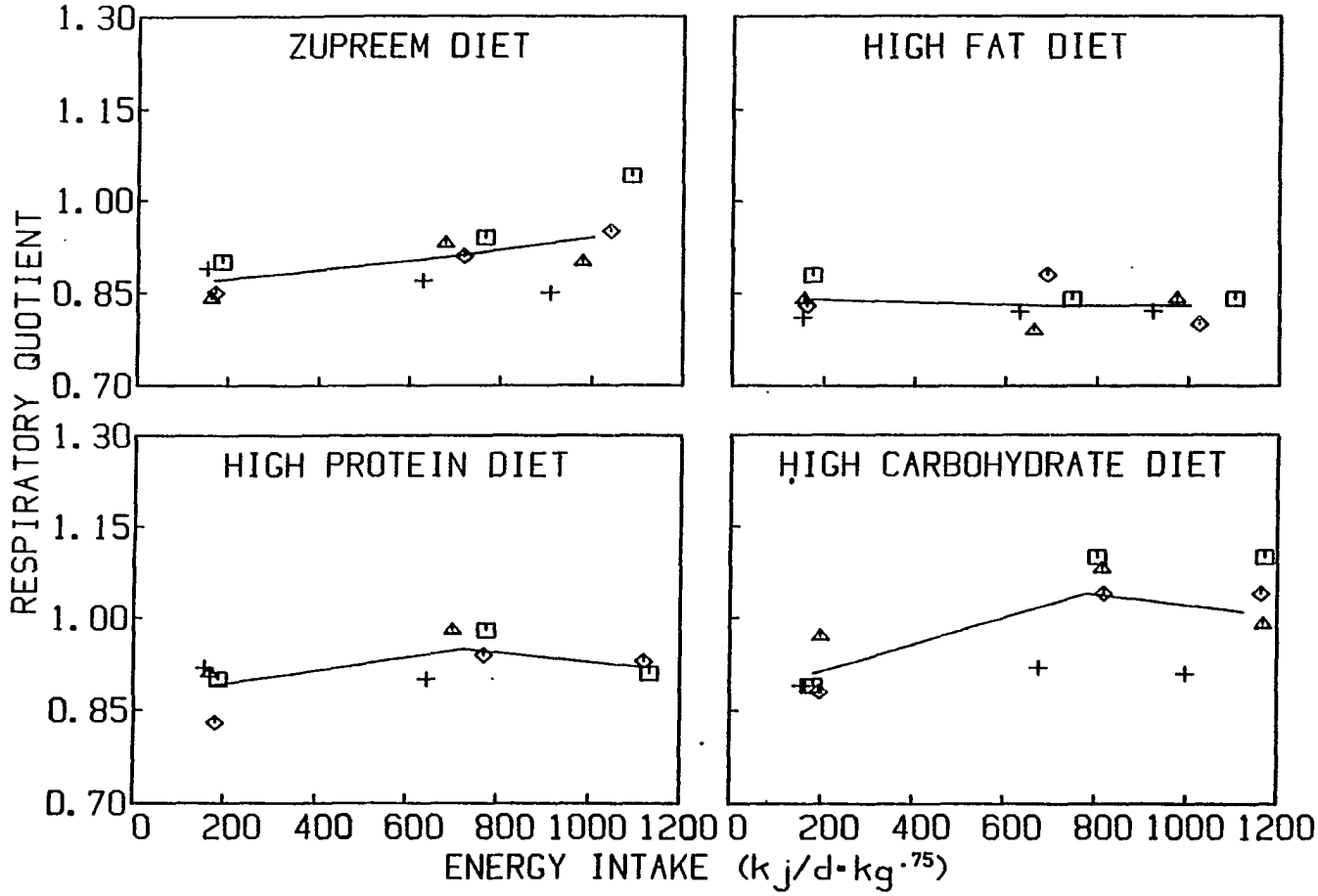

Figure 8. Preprandial respiratory quotient of four arctic fox in relation to diet and plane of nutrition.

Table 10. Preprandial respiratory quotient of four arctic fox in relation to diet and plane of nutrition. Values represent the mean for the 6 hours preceeding feeding.

kj/d	ZUP[1]	FAT[2]	PRO[3]	CHO[4]	Significance	
450[A]	0.87 ±0.01	0.84 ±0.01	0.89 ±0.02	0.91§ ±0.02	2 vs 4	P<0.05
1800[B]	0.91 ±0.02	0.83 ±0.02	0.95 ±0.02	1.04§ ±0.04	1 vs 2 2 vs 3,4 4 vs 1,3	P<0.025 P<0.001 P<0.001,0.01
2700[C]	0.94 ±0.04	0.83 ±0.01	0.92‡ ±0.01	1.01§ ±0.04	1 vs 2 2 vs 3 4 vs 1,2 4 vs 3	P<0.002 P<0.025 P<0.05,0.001 P<0.025
Significance:	A vs C P<0.05	NS	NS	A vs B P<0.001 A vs C P<0.005		

mean values ± sem, n=4; ‡ n=2 ; § significantly less than postprandial value (P<0.001).

The equations for MP and HP were not significantly different from one another and the data were fitted to one regression (Figure 8):

MPHP PRRQ = 1.04 - 0.228 FE r^2=0.562
(0.038) P<0.0001 n=30

The relationship was much stronger for dietary fat energy and RQ than for protein, especially at LP. A low fat and protein diet would be correspondingly very high in carbohydrate. The highest preprandial RQ at all planes of nutrition was noted for the CHO diet (MP 1.04±0.04), while the lowest was noted for the FAT diet (MP 0.91±0.02).

The postprandial RQ (PORQ) and diet composition relationship (Figure 9; Table 11) was stronger, as seen in the following regressions.

LP PORQ = 1.16 - 0.375 FE - 0.181 PE r^2=0.831
(0.050) (0.055) P<0.0001 n=15

MP PORQ = 1.32 - 0.498 FE - 0.358 PE r^2=0.774
(0.078) (0.096) P<0.0001 n=15

HP PORQ = 1.32 - 0.516 FE - 0.313 PE r^2=0.769
(0.094) (0.123) P<0.0014 n=12

The above equations were not significantly different from one another and the data were fitted to a single regression encompassing all diet planes (AP).

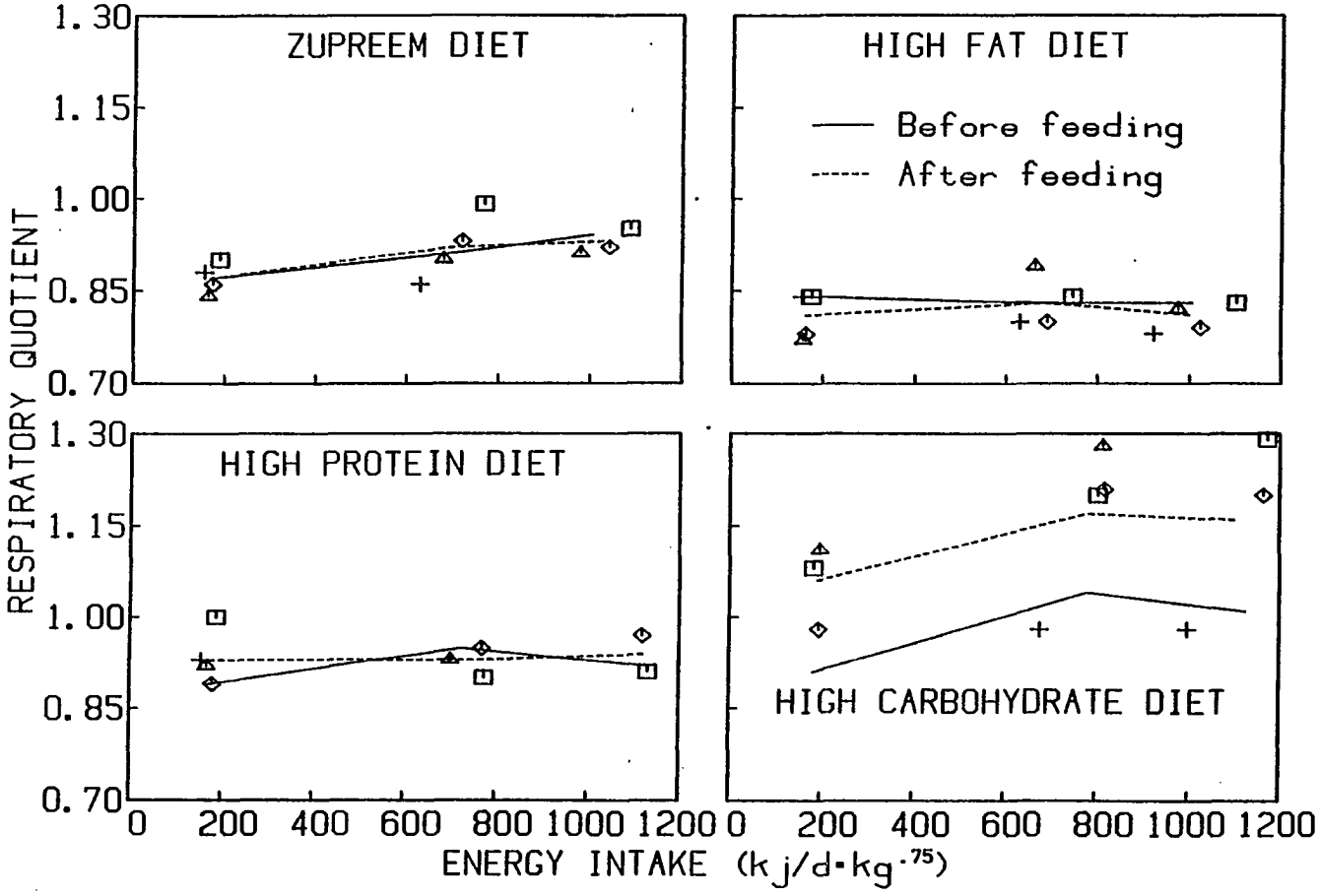

Figure 9. Postprandial respiratory quotient of four arctic fox in relation to diet and plane of nutrition.

Table 11. Postprandial respiratory quotient of four arctic fox in relation to diet and plane of nutrition. Values represent the mean for the period between 2 and 10 hours after feeding.

kj/d	ZUP[1]	FAT[2]	PRO[3]	CHO[4]	Significance	
450[A]	0.87 ±0.01	0.81 ±0.02	0.93 ±0.02	1.06†§ ±0.04	1 vs 2,3 2 vs 3,4 4 vs 1,3	P<0.025 P<0.001 P<0.001
1800[B]	0.92 ±0.03	0.83 ±0.02	0.93† ±0.02	1.17§ ±0.07	2 vs 1,3 4 vs 1,2,3	P<0.002 P<0.001
2700[C]	0.93† ±0.01	0.81 ±0.01	0.94‡ ±0.03	1.16†§ ±0.09	2 vs 1,3,4 4 vs 1,3	P<0.001 P<0.001
Significance:	A vs B,C P<0.05	NS	NS	A vs B P<0.001 A vs C P<0.005		

mean values ± sem, n=4; † n=3 ; ‡ n=2 ; § significantly greater than preprandial value (P<0.001).

AP PORQ = 1.27 - 0.470 FE - 0.294 PE r^2=0.750
(0.043) (0.052) P<0.0001 n=42

Regression analyses indicated significant relationships at all planes of intake between the change in RQ after feeding (CHRQ) and dietary energy composition (Figure 10; Table 12):

LP CHRQ = 24.2 - 30.0 FE - 16.2 PE r^2=0.768
(4.8) (5.3) P<0.0002 n=15

MP CHRQ = 20.3 - 19.5 FE - 26.3 PE r^2=0.553
(6.2) (7.6) P<0.008 n=15

HP CHRQ = 22.1 - 26.3 FE - 20.7 PE r^2=0.786
(4.6) (6.0) P<0.001 n=12

These regressions were not significantly different from one another and were combined into one equation:

AP CHRQ = 21.9 - 25.0 FE - 20.0 PE r^2=0.619
(3.2) (3.8) P<0.0001 n=42

In the CHO group, RQ increased at all levels of intake. RQ of the ZUP and CHO groups increased significantly between planes of nutrition, thus lowest energy intake resulted in the lowest RQ's in both the pre- and postprandial state.

1.3.4 Urinary Nitrogen Excretion

Urinary nitrogen excretion (UNE, g/d) was directly related to the contribution of protein to dietary energy

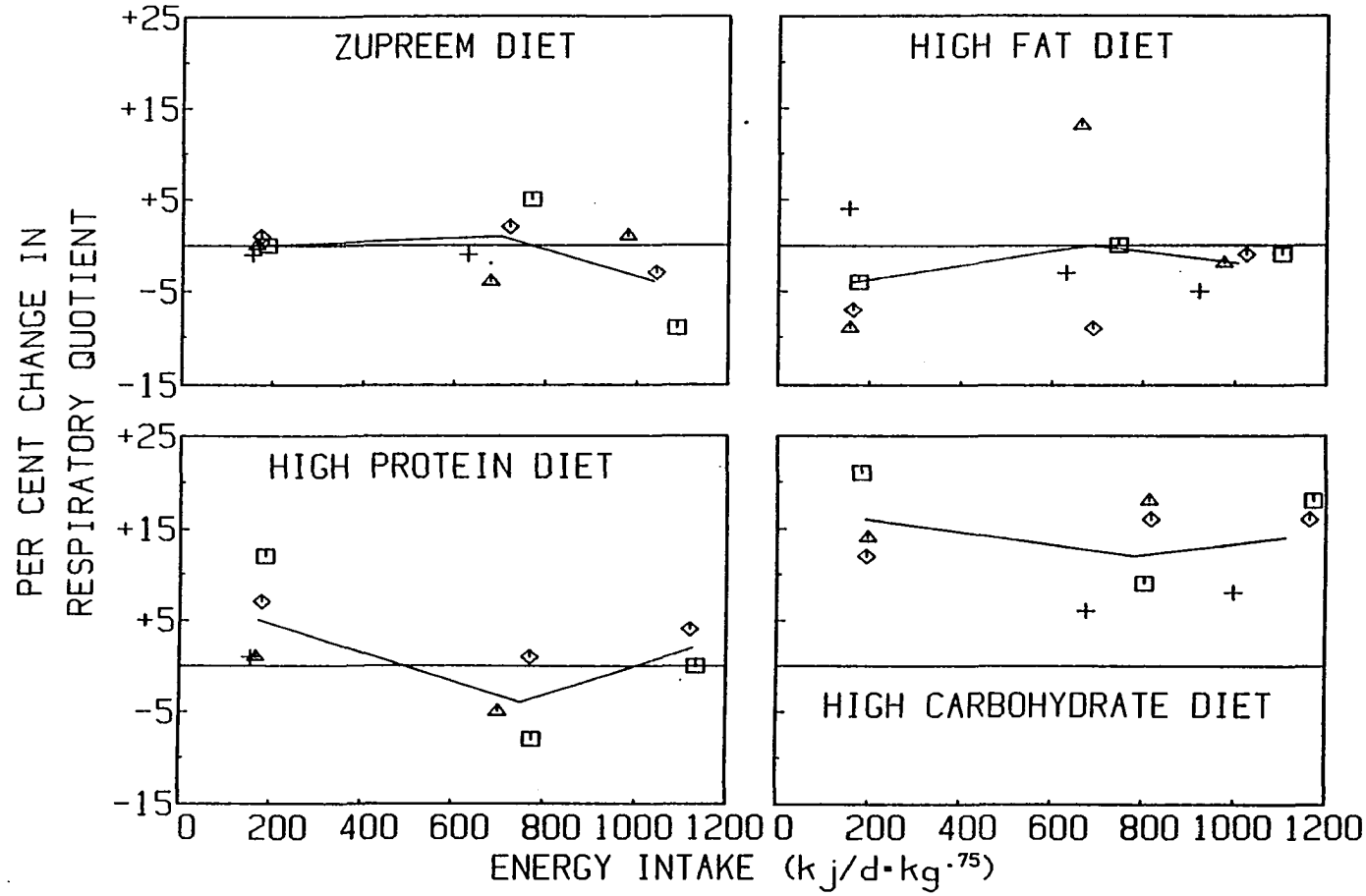

Figure 10. Change in respiratory quotient in four arctic fox after feeding in relation to diet and plane of nutrition.

Table 12. Per cent change from pre- to postprandial respiratory quotient of four arctic fox in relation to diet and plane of nutrition.

kj/d	ZUP[1]	FAT[2]	PRO[3]	CHO[4]	Significance	
450[A]	0	-4	5	16†	2 vs 3,4	P<0.05,0.001
	±1	±3	±3	±3	4 vs 1,3	P<0.005,0.05
1800[B]	1	0	-4†	12	4 vs 1,2	P<0.01
	±2	±5	±3	±3	3 vs 4	P<0.002
2700[C]	-4†	-2	2‡	14†	4 vs 1,2	P<0.005
	±3	±1	±2	±3	4 vs 3	P<0.05
Significance:	NS	NS	A vs B P<0.05	NS		

mean values ± sem, n=4; † n=3 ; ‡ n=2

(Figure 11; Table 13). The regressions describing this relationship did not include the FE coefficient as it was insignificant.

LP UNE = 0.211 + 2.88 PE r^2=0.847
(0.35) P<0.0001 n=14

MP UNE = -0.105 + 12.6 PE r^2=0.962
(0.7) P<0.0001 n=14

HP UNE = -0.31 + 18.5 PE r^2=0.896
(2.4) P<0.0001 n=9

A more significant relationship existed if the diets were described in terms of nitrogen intake (NI, g/d).

UNE = 0.0803 + 0.673 NI r^2=0.949
0.026 P<0.0001 n=38

Accordingly, UNE for the PRO diet significantly exceeded that of the other groups at LP and MP intake level (Table 13). Lost samples eliminated data for the PRO diet at the HP intake. UNE was lowest for the FAT diet at all planes of nutrition; protein intake contributed least to total dietary energy at all levels of intake for this diet (Table 3). Although UNE increased directly with plane of energy intake within the PRO, CHO, and ZUP diets, no significant relationship was found in the FAT group.

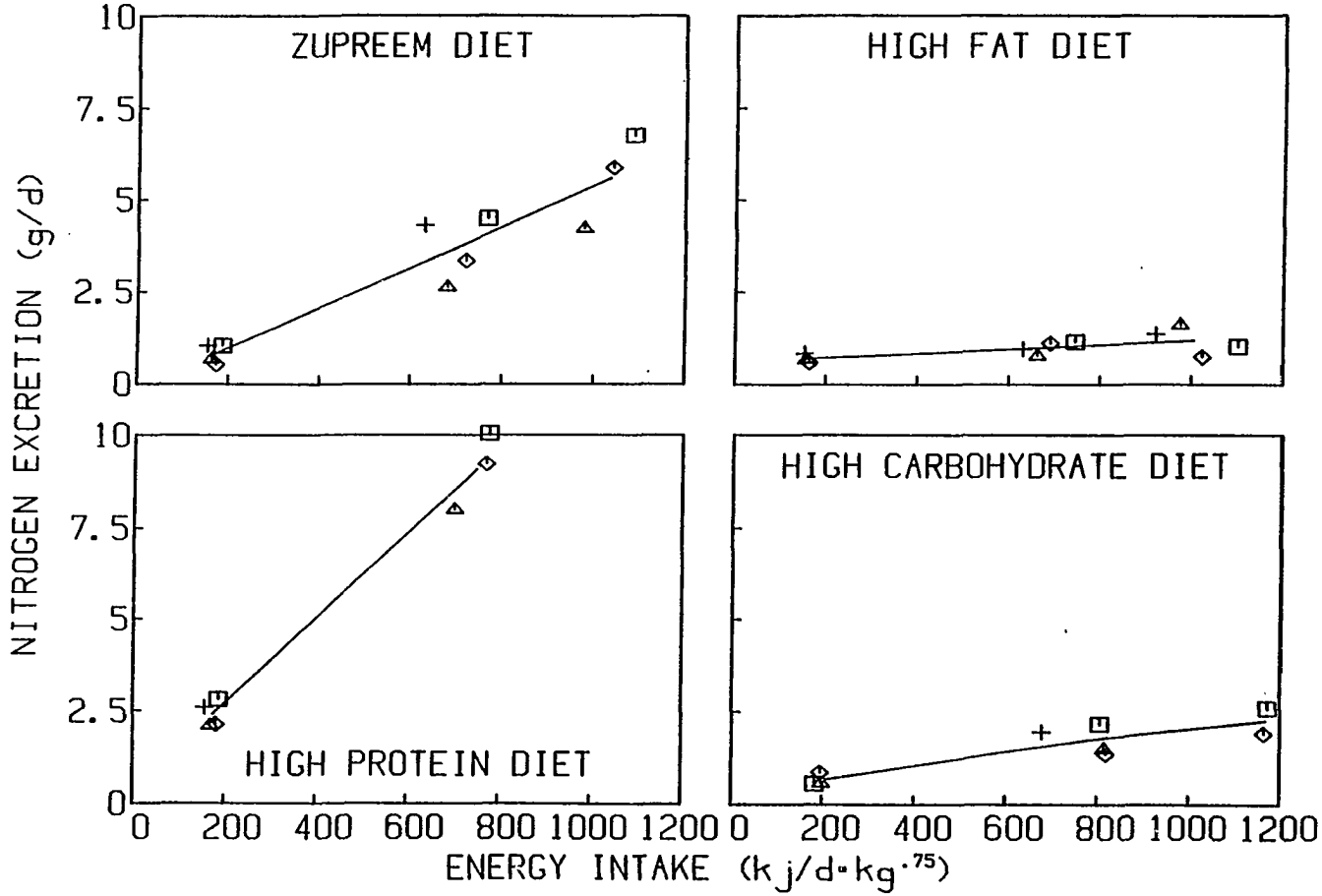

Figure 11. Urinary nitrogen excretion of four arctic fox in relation to diet and plane of nutrition.

Table 13. Urinary nitrogen excreted (g/d) by four arctic fox in relation to diet and plane of nutrition.

kj/d	ZUP[1]	FAT[2]	PRO[3]	CHO[4]	Significance	
450[A]	0.80 ±0.13	0.69† ±0.08	2.41 ±0.17	0.65† ±0.10	3 vs 1,2,4	P<0.001
1800[B]	3.69 ±0.44	0.99 ±0.09	9.08† ±0.59	1.74 ±0.19	3 vs 1,2,4 1 vs 2,4 2 vs 4	P<0.001 P<0.001 P<0.05
2700[C]	5.60† ±0.75	1.17 ±0.19	§	2.25‡ ±0.34	1 vs 2,4 4 vs 2	P<0.001 P<0.025
Significance:	A vs B,C B vs C P<0.001	NS	A vs B P<0.001	A vs B P<0.01 A vs C P<0.005		

mean values ± sem, † n=3 ; ‡ n=2 ; § data not available

1.3.5 Metabolic mixture

1.3.5.1 --Energy expenditure--

Energy expenditure (EE, $kj/d \cdot kg^{0.75}$) was inversely related to energy intake (EI, $kj/d \cdot kg^{0.75}$) in the 2 diets high in fat, FAT and ZUP, while a direct relationship existed for the diet lowest in fat, the CHO diet. No significant regression was noted for the PRO diet.

ZUP EE = 266 - 0.0397 EI r^2=0.366
0.0170 P<0.049 n=11

FAT EE = 337 - 0.0542 EI r^2=0.444
0.0202 P<0.025 n=11

CHO EE = 196 + 0.0274 EI r^2=0.454
0.0114 P<0.047 n=9

FAT was associated with the highest energy expenditures across all planes of energy intake (MP 300±13), while the least EE was observed in the CHO group (MP 213±7) (Figure 12; Table 14). There was little change in these comparisons if energy expenditure was expressed on a per fox basis.

Contribution of fat and protein to dietary energy were directly related to EE at LP and MP, although the protein relationship was much weaker than for fat and was omitted from the regressions at LP and MP. At HP, the direct effect of fat on EE remained, albeit less strong,

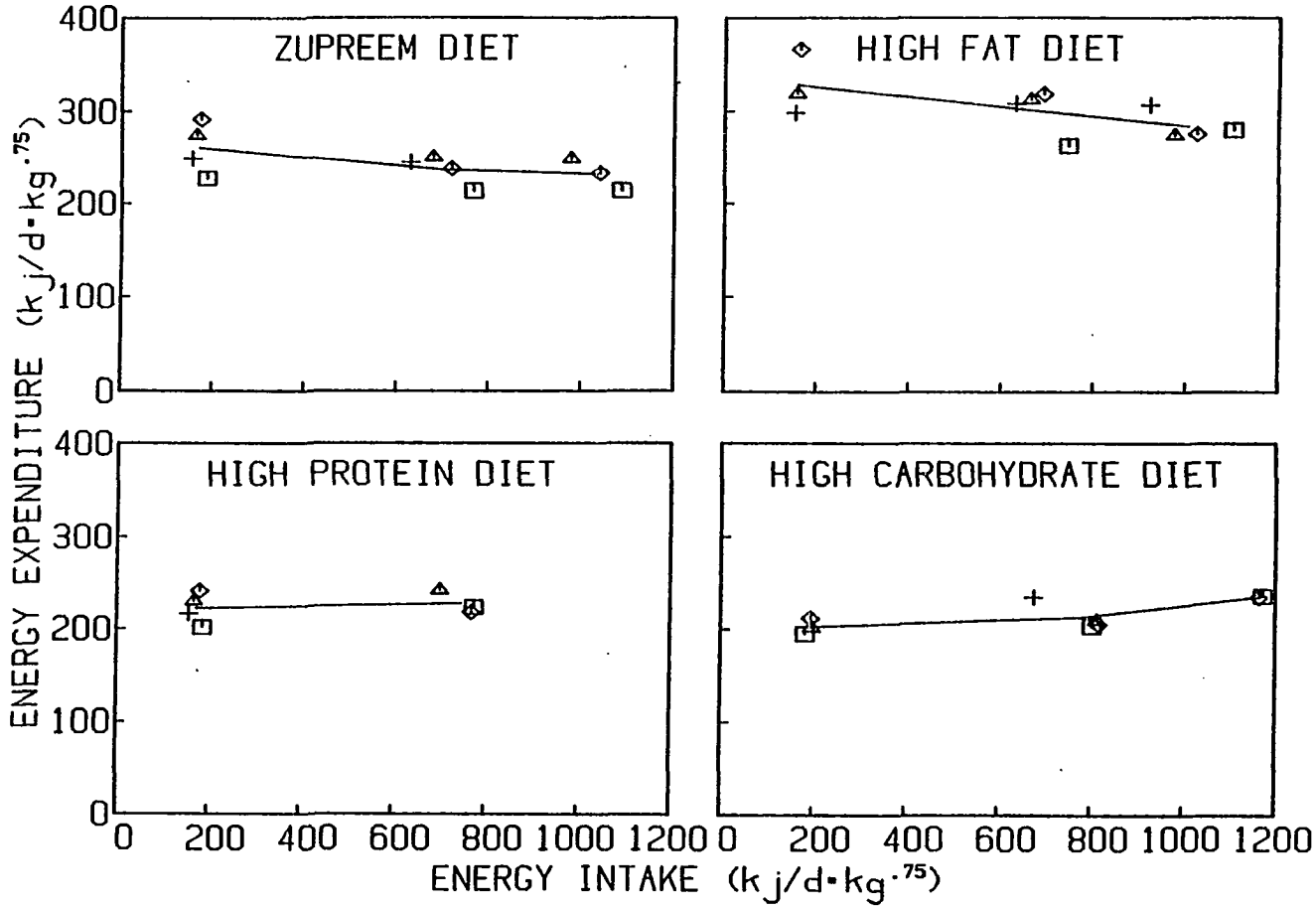

Figure 12. Energy expenditure of four arctic fox in relation to diet and plane of nutrition.

Table 14. Energy expenditure (kj/d-kg$^{0.75}$) of four arctic fox in relation to diet and plane of nutrition. Values calculated from daily urinary nitrogen excretion, oxygen consumption, and respiratory quotient.

kj/d	ZUP[1]	FAT[2]	PRO[3]	CHO[4]	Significance	
450[A]	260 ±14	328† ±20	222 ±9	203† ±5	2 vs 1,3,4 1 vs 3,4 3 vs 4	P<0.001 P<0.001 P<0.05
1800[B]	236 ±8	300 ±13	227† ±7	213 ±7	2 vs 1,3,4 1 vs 4	P<0.001 P<0.025
2700[C]	231† ±10	284 ±8	§	235‡ ±1	2 vs 1,4	P<0.001
Significance:	A vs B P<0.025 A vs C P<0.01	A vs B P<0.01 A vs C P<0.001	NS	A vs C P<0.025 B vs C P<0.05		

mean values ± sem, † n=3 ; ‡ n=2 ; § data not available

while the effect of protein was reversed and elicited a strong negative effect on EE.

LP EE = 173 + 160 FE r^2=0.786
(24) P<0.0001 n=14

MP EE = 189 + 108 FE r^2=0.683
(20) P<0.0001 n=15

HP EE = 246 + 56.8 FE - 157 PE r^2=0.820
(19.1) (48) P<0.0058 n=9

The LP and MP regressions were not significantly different from one another, and were combined into one regression equation:

LPMP EE = 182 + 131 FE r^2=0.709
(16) P<0.0001 n=29

1.3.5.2 --Protein--

Since protein metabolized is defined as the nitrogen excreted multiplied by the constant 6.25, protein oxidized by the fox followed the same pattern as UNE (Figure 13; Table 15). Protein oxidation was directly related to the protein contribution to dietary energy, as seen in the following regressions, which were all significantly different from one another:

LP PRO(g/d) = 1.32 + 18.0 PE r^2=0.847
(2.2) P<0.0001 n=14

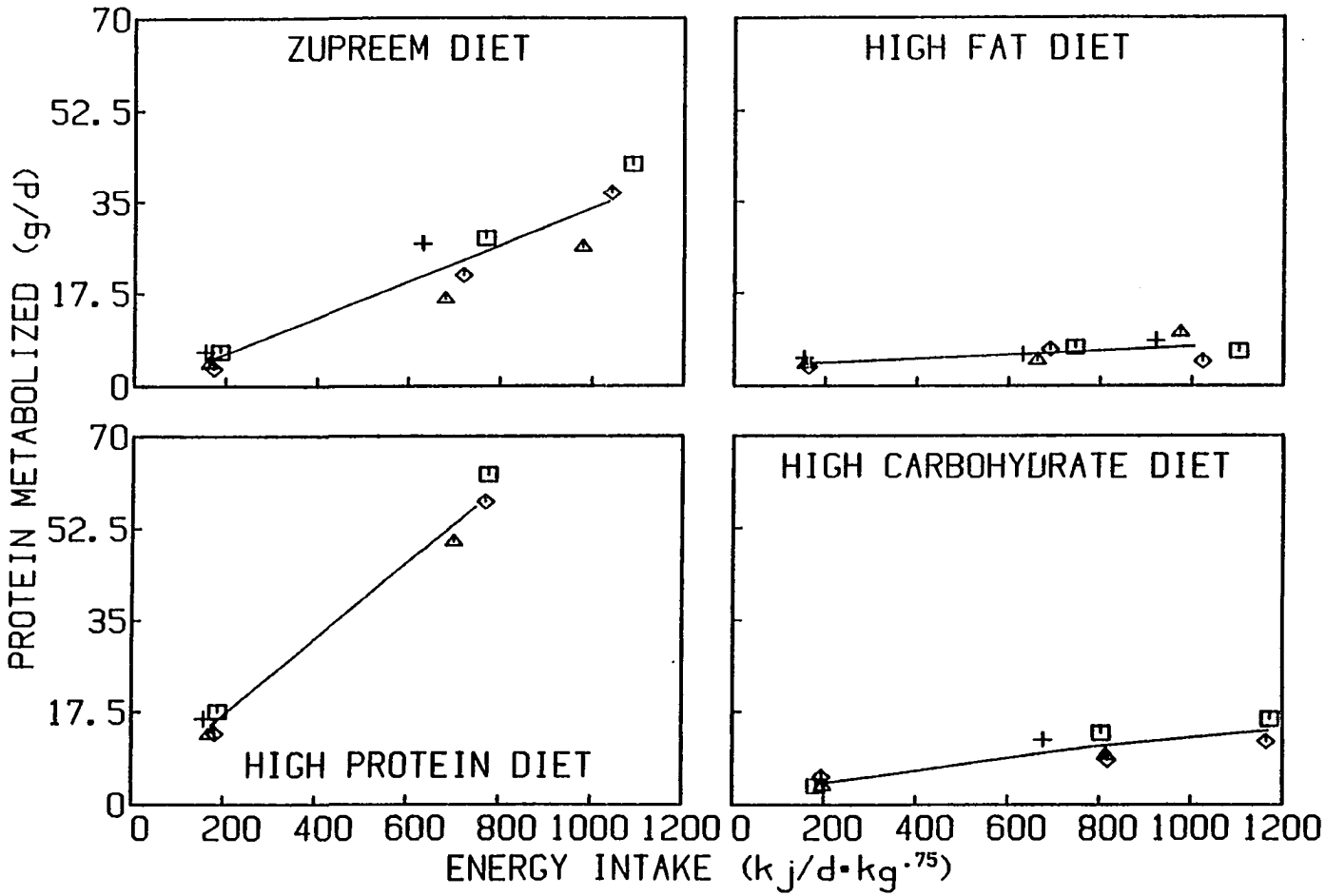

Figure 13. Protein metabolized by four arctic fox in relation to diet and plane of nutrition.

Table 15. Protein metabolized (g/d) by four arctic fox in relation to diet and plane of nutrition.

kj/d	ZUP[1]	FAT[2]	PRO[3]	CHO[4]	Significance	
450[A]	5.0 ±0.8	4.3† ±0.5	15.1 ±1.1	4.1† ±0.6	3 vs 1,2,4	P<0.001
1800[B]	23.1 ±2.7	6.2 ±0.5	56.8† ±3.7	10.8 ±1.2	2 vs 4 1 vs 2,3,4 3 vs 2,4	P<0.05 P<0.001 P<0.001
2700[C]	35.0† ±4.7	7.3 ±1.2	§	14.1‡ ±2.2	2 vs 4 1 vs 2,4	P<0.025 P<0.001
Significance:	A vs B,C B vs C P<0.001	NS	A vs B P<0.001	A vs B P<0.01 A vs C P<0.005		

mean values ± sem, † n=3 ; ‡ n=2 ; § data not available

MP PRO(g/d) = -0.657 + 78.8 PE r^2=0.962
(4.4) P<0.0001 n=15

HP PRO(g/d) = -1.95 + 115 PE r^2=0.896
(15) P<0.0001 n=9

When protein oxidation (g/d) was regressed against protein intake (PI) (g/d) for each plane of nutrition, the resulting direct relationships were not significantly different from one another, and were combined into one equation.

AP PRO(g/d) = 0.00468 + 0.718 PI r^2=0.947
(0.028) P<0.0001 n=38

The contribution of protein oxidation to energy expenditure (PRO%EE) was directly related to dietary protein energy (Table 3), and inversely related to dietary fat energy. Most of the variance in the following regressions was contributed by the protein content of the diets.

LP PRO%EE = 4.44 + 45.1 PE r^2=0.883
(5.7) P<0.0001 n=14

MP PRO%EE = 17.0 + 84.1 PE r^2=0.756
(13.3) P<0.0001 n=15

HP PRO%EE = 33.8 - 29.4 FE + 107 PE r^2=0.908
(7.9) (20) P<0.0008 n=9

Thus, the effect of dietary fat depended on whether an animal was gaining weight at an appreciable rate.

Daily nitrogen intake (NI) directly affected the protein oxidation proportion of energy expenditure.

ZUP PRO%EE = 7.95 + 5.62 NI (r^2=0.754
(1.07) P<0.0005 n=11

PRO PRO%EE = 26.5 + 3.71 NI (r^2=0.833
(0.74) P<0.004 n=7

FAT PRO%EE = 6.97 + 4.69 NI (r^2=0.440
(1.76 P<0.026 n=11

CHO PRO%EE = 11.2 + 10.4 NI (r^2=0.736
(2.4) P<0.0031 n=9

Regressions calculated for each plane, including all diets, indicated a direct relationship between PRO%EE and NI.

LP PRO%EE = 5.04 + 9.72 NI (r^2=0.828
(1.28) P<0.0001 n=14

MP PRO%EE = 18.0 + 4.57 NI (r^2=0.769
(0.73) P<0.0001 n=15

HP PRO%EE = 12.7 + 4.60 NI (r^2=0.668
(1.22) P<0.0071 n=9

These equations were not significantly different from one another, and so were combined into one regression.

AP PRO%EE = 13.3 + 5.02 NI (r^2=0.760
(0.47) P<0.0001 n=38

The greatest contribution of protein oxidation to total energy expenditure was found in the PRO group (LP 38.6%±0.8), while the least was in the FAT group (LP 8.3%±1.2) (Figure 14; Table 16). Values for the ZUP and CHO diets were intermediate. The percent contribution of protein to energy expenditure generally doubled from LP to MP, but changed little from MP to HP.

1.3.5.3 --Fat--

Fat oxidation (g/d) by the fox was directly related to the percent contribution of fat to total dietary energy, and inversely related to that of protein content. The inverse relationship to protein content was appreciably weaker than that for fat content at MP and HP, and was insignificant at LP.

LP FAT (g/d) = -5.38 + 19.9 FE (r^2=0.856
(2.4) P<0.0001 n=14

MP FAT (g/d) = -10.1 + 22.6 FE - 14.9 PE (r^2=0.895
(3.3) (4.1) P<0.0001 n=15

HP FAT (g/d) = -13.2 + 29.7 FE - 40.4 PE (r^2=0.914
(4.9) (12.4) P<0.0006 n=9

Similar relationships were evident if fat oxidation was regressed against absolute amounts of fat intake (FI) (g/d).

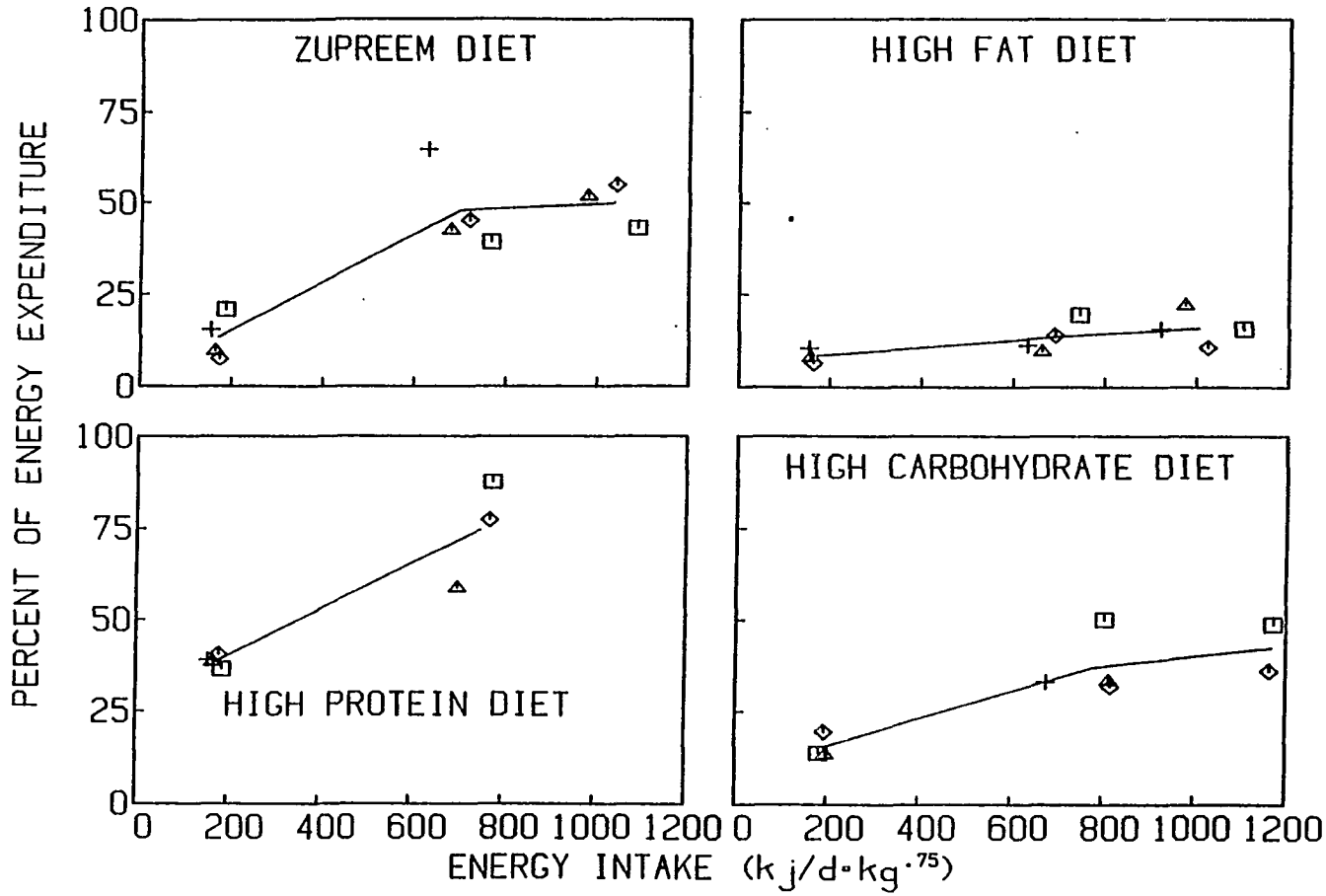

Figure 14. Contribution of protein oxidation to energy use by four arctic fox in relation to diet and plane of nutrition.

Table 16. Percentage of total energy expenditure provided by protein oxidation in four arctic fox. Values calculated from daily urinary nitrogen excretion, oxygen consumption, and carbon dioxide production.

kj/d	ZUP[1]	FAT[2]	PRO[3]	CHO[4]	Significance	
450[A]	13.3 ±3.0	8.3† ±1.2	38.6 ±0.8	15.5† ±2.0	3 vs 1,2 3 vs 4	P<0.005 P<0.025
1800[B]	47.7 ±5.7	13.6 ±2.1	74.5† ±8.5	37.2 ±4.3	3 vs 2,4 1 vs 2,3 2 vs 4	P<0.001 P<0.005 P<0.025
2700[C]	49.7† ±3.5	16.0 ±2.3	§	42.7‡ ±6.4	2 vs 1,4	P<0.005
Significance:	A vs B,C P<0.005,0.001	NS	A vs B P<0.001	A vs B,C P<0.025,0.005		

mean values ± sem, † n=3 ; ‡ n=2 ; § data not available

LP FAT (g/d) = -5.41 + 1.68 FI (r^2=0.856
(0.20) P<0.0001 n=14

MP FAT (g/d) = -17.3 + 0.596 FI (r^2=0.781
(0.088) P<0.0001 n=15

HP FAT (g/d) = -23.2 + 0.484 FI (r^2=0.758
(0.103) P<0.0022 n=9

At the LP, the FAT group exhibited the greatest rate of fat oxidation (13.4±1.2), while the PRO and CHO groups showed a low level of fat synthesis (PRO 0.3±1.3) (Figure 15; Table 17). The ZUP group was intermediate, oxidizing fat at half the rate of the FAT group. Fat metabolism was inversely related to plane of energy intake. ZUP, CHO, and PRO groups synthesized fat at increasingly greater rate as they were fed at higher planes of nutrition (CHO LP 0.7±1.0; MP 7.4±2.1; HP 12.6±2.4). Although the FAT group never synthesized fat, it did oxidize less at MP and HP than at LP.

The percentage of total energy usage provided by fat oxidation was also directly related to the contribution of fat to dietary energy (Figure 16; Table 18).

LP FAT%EE = -14.3 + 79.2 FE (r^2=0.873
(8.7) P<0.0001 n=14

MP FAT%EE = -17.9 + 59.9 FE (r^2=0.659
(11.9) P<0.0002 n=15

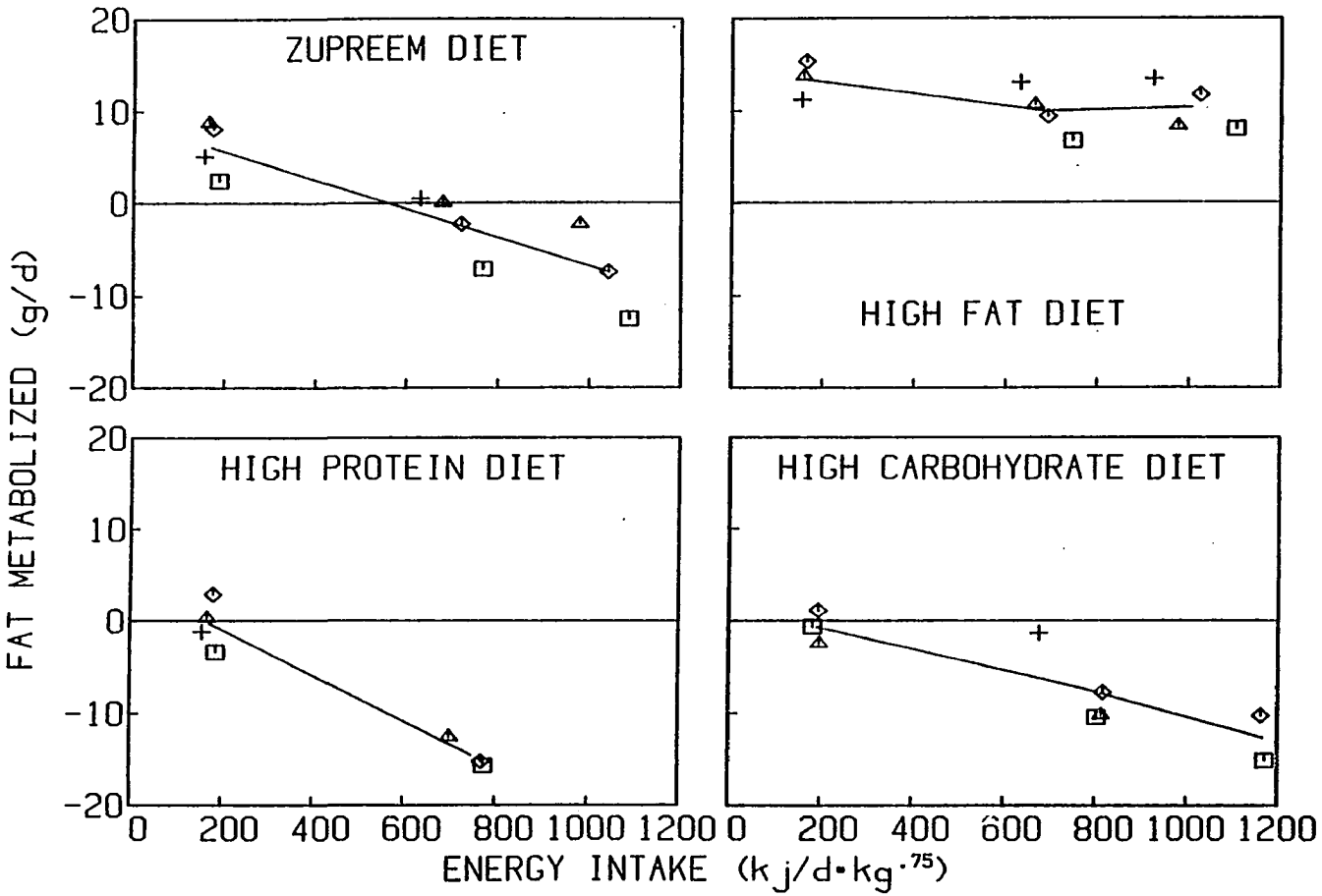

Figure 15. Fat metabolized by four arctic fox in relation to diet and plane of nutrition. Negative values indicate synthesis.

Table 17. Fat metabolized (g/d) by four arctic fox in relation to diet and plane of nutrition. Negative values indicate fat synthesis.

kj/d	ZUP[1]	FAT[2]	PRO[3]	CHO[4]	Significance	
450[A]	6.1 ±1.5	13.4† ±1.2	-0.3 ±1.3	-0.7† ±1.0	2 vs 1,3,4 1 vs 3,4	P<0.001 P<0.001
1800[B]	-2.2 ±1.8	9.9 ±1.3	-14.5† ±1.0	-7.4 ±2.1	2 vs 1,3,4 1 vs 3,4 3 vs 4	P<0.001 P<0.001 P<0.001
2700[C]	-7.4† ±3.0	10.3 ±1.3	§	-12.6‡ ±2.4	2 vs 1,4 1 vs 4	P<0.001 P<0.005
Significance:	A vs B,C P<0.001 B vs C P<0.005	A vs B,C P<0.025	A vs B P<0.001	A vs B,C P<0.001 B vs C P<0.005		

mean values ± sem, † n=3 ; ‡ n=2 ; § data not available

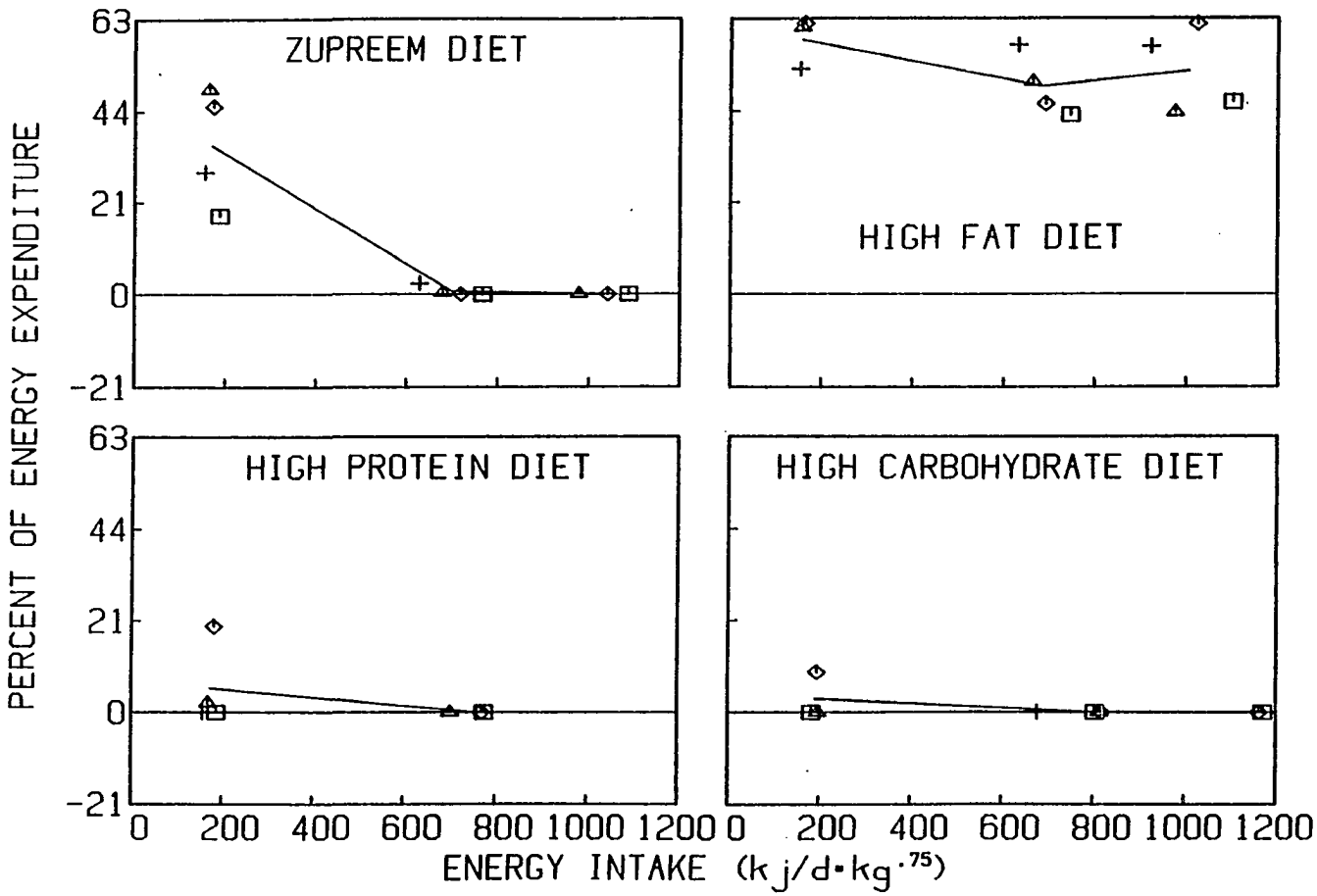

Figure 16. Contribution of fat oxidation to energy use by four arctic fox in relation to diet and plane of nutrition.

Table 18. Percentage of total energy expenditure provided by fat oxidation in four arctic fox. Values calculated from daily urinary nitrogen excretion, oxygen consumption, and carbon dioxide production.

kj/d	ZUP[1]	FAT[2]	PRO[3]	CHO[4]	Significance	
450[A]	34.0 ±6.8	58.1† ±3.3	5.5 ±4.8	3.1† ±3.3	2 vs 1,3,4 1 vs 3,4	P<0.001 P<0.001
1800[B]	0.7 ±0.6	47.4 ±3.5	0.0† ±0.0	0.0 ±0.0	2 vs 1,3,4	P<0.001
2700[C]	0.0† ±0.0	50.9 ±4.9	§	0.0‡ ±0.0	2 vs 1,4	P<0.001
Significance:	A vs B,C P<0.001	A vs B,C P<0.05	A vs B P<0.05	NS		

mean values ± sem, † n=3 ; ‡ n=2 ; § data not available

HP FAT%EE = +7.16 + 61.4 FE - 143 PE r^2=0.953
(9.03) (23) P<0.0001 n=9

As energy intake increased, the effect of fat content on the regression weakened, while the negative effect of protein content became stronger. Fat oxidation contributed little, if at all, to total energy expenditure, when fat synthesis occurred.

1.3.5.4 --Carbohydrate--

Oxidation of carbohydrate (g/d) was negatively influenced by the contribution of fat and protein to the total energy content of the diet. This relationship was not significant at LP.

MP CHO (g/d) = 47.2 - 27.2 FE - 42.8 PE r^2=0.835
(4.8) (5.8) P<0.0001 n=15

HP CHO (g/d) = 64.4 - 51.0 FE - 43.8 PE r^2=0.971
(3.6) (9.0) P<0.0001 n=9

The direct relationship between carbohydrate oxidation (g/d) and carbohydrate intake (CI) (g/d) was significant at all planes of energy intake. Since the equations were not significantly different from one another, they were combined.

AP CHO (g/d) = 16.3 + 0.292 CI r^2=0.790
(0.025) P<0.0001 n=36

The CHO group's carbohydrate oxidation was significantly greater than that of the other groups at all planes of energy intake (CHO HP 47.5±3.5, FAT HP 14.9±1.1) (Figure 17; Table 19). As the plane of nutrition increased, there was a corresponding increase in carbohydrate oxidation, while the opposite was true in the PRO group. No such changes were seen in the other groups, although there was a slightly significant decrease in the FAT group's carbohydrate oxidation as the plane of nutrition increased from MP to HP.

At the LP and MP, the percent contribution of carbohydrate oxidation to total energy usage was also inversely related to the dietary fat and protein energy. When an animal was losing weight, fat content was a more important determinant than protein, while the reverse was true when a fox was gaining weight on MP. At the HP the effect of protein was positive.

LP CHO%EE = 101 - 66.6 FE - 36.3 PE r^2=0.782
(10.6) (11.4) P<0.0002 n=14

MP CHO%EE = 77.2 - 77.2 FE - 53.8 PE r^2=0.508
(12.9) (15.8) P<0.0142 n=15

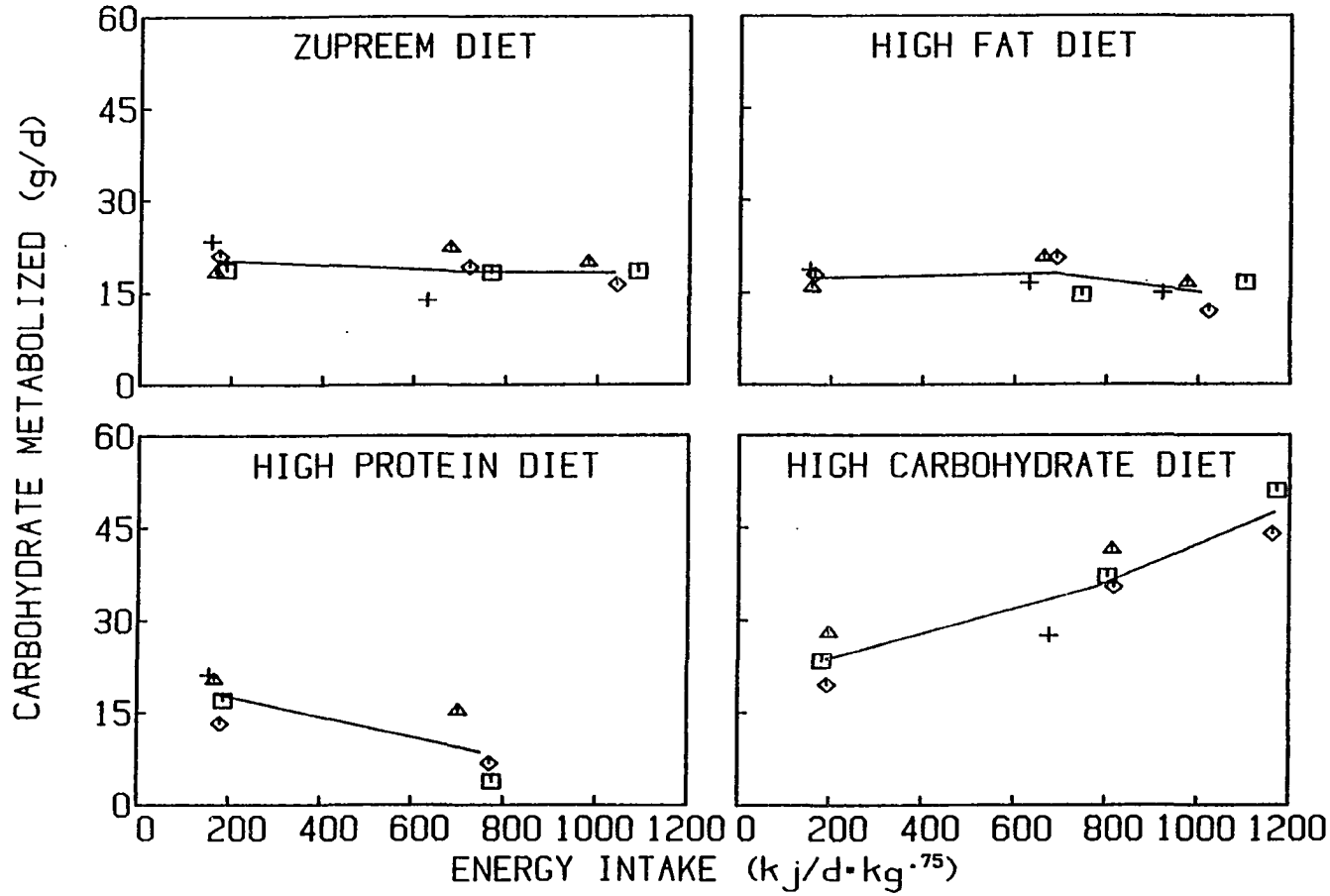

Figure 17. Carbohydrate metabolized by four arctic fox in relation to diet and plane of nutrition.

Table 19. Carbohydrate metabolized (g/d) by four arctic fox in relation to diet and plane of nutrition.

kj/d	ZUP[1]	FAT[2]	PRO[3]	CHO[4]	Significance	
450[A]	20.3 ±1.2	17.5† ±0.8	17.9 ±1.8	23.6† ±2.4	4 vs 2,3 4 vs 1	P<0.005 P<0.05
1800[B]	18.4 ±1.8	18.1 ±1.5	8.6† ±3.4	35.4 ±2.9	4 vs 1,2,3 3 vs 1,2	P<0.001 P<0.001
2700[C]	18.2† ±1.0	14.9 ±1.1	§	47.5‡ ±3.5	1 vs 2 4 vs 1,2	P<0.05 P<0.001
Significance:	NS	B vs C P<0.05	A vs B P<0.001	A vs B,C P<0.001 B vs C P<0.001		

mean values ± sem, † n=3 ; ‡ n=2 ; § data not available

HP CHO%EE = 59.0 - 36.1 FE (9.5) r^2=0.676 P<0.0066 n=9

The carbohydrate oxidation contribution in the CHO group was highest of all groups at the LP (81.4%±5.1) (Figure 18; Table 20). As the plane of nutrition increased, there was a concomitant decrease in the percentage of carbohydrate utilized for energy expenditure. This was a reflection of the CHO group's increasing synthesis of fat from carbohydrate and its reliance on protein oxidation. This latter phenomenon was seen dramatically in the PRO group as the plane of energy intake increased from LP to MP. No change in carbohydrate contribution to energy expenditure across planes of nutrition was seen in the ZUP and FAT groups. Although the ZUP group exhibited increasing protein oxidation from LP to MP and HP, there seemed not to be enough fat synthesis to negatively affect the carbohydrate oxidation's proportion of energy expenditure. The FAT group showed little change in protein, fat, or carbohydrate metabolism across planes of nutrition.

1.3.5.5 --Metabolic water--

The four diet groups differed significantly in their metabolically produced water (g/d) (Figure 19; Table 21). At the LP, the FAT group was the highest producer (LP

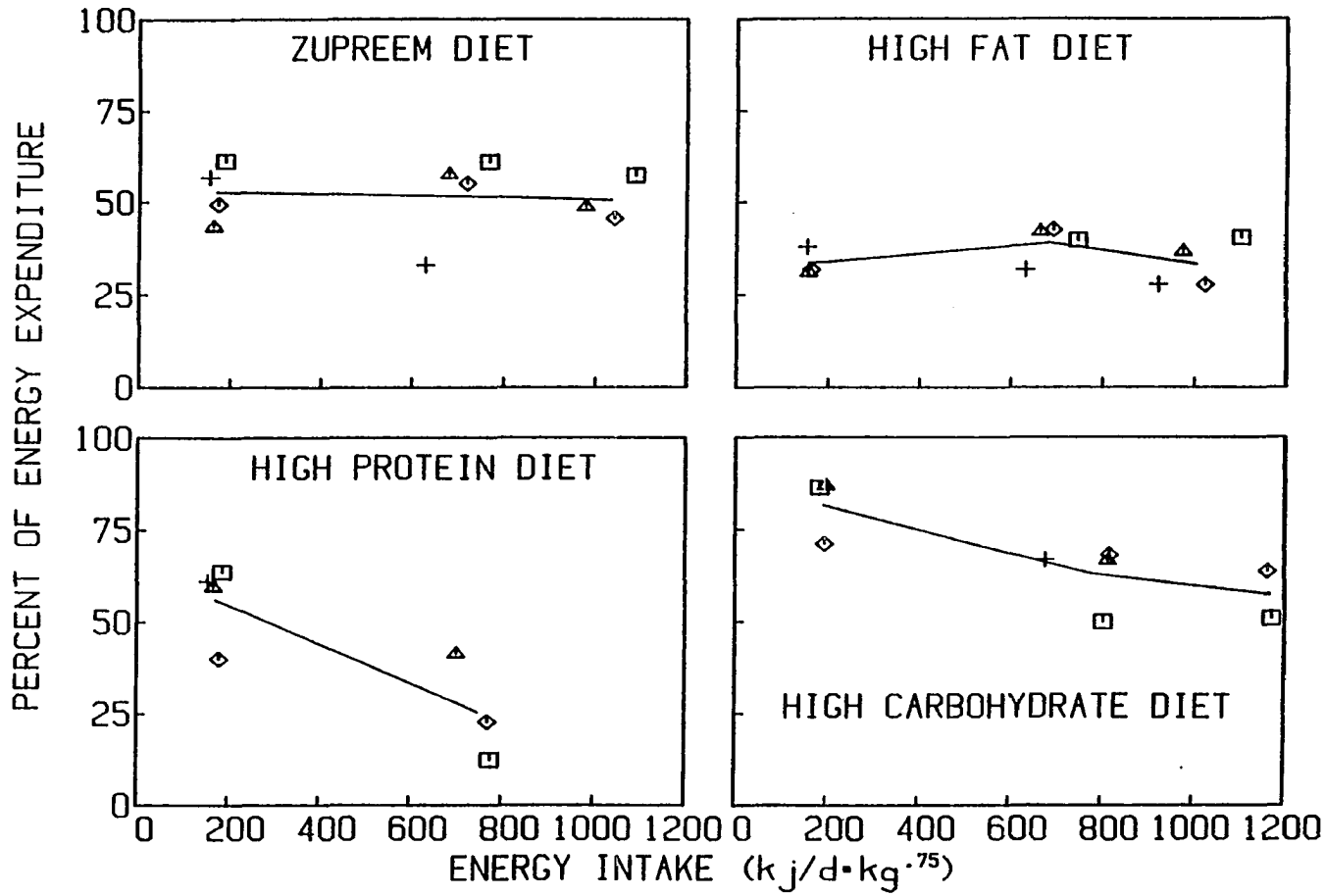

Figure 18. Contribution of carbohydrate oxidation to energy use by four arctic fox in relation to diet and plane of nutrition.

Table 20. Percentage of total energy expenditure provided by carbohydrate oxidation in four arctic fox. Values calculated from daily urinary nitrogen excretion, oxygen consumption, and carbon dioxide production.

kj/d	ZUP[1]	FAT[2]	PRO[3]	CHO[4]	Significance	
450[A]	52.6 ±4.0	33.6† ±2.2	55.9 ±5.4	81.4† ±5.1	2 vs 1,3 4 vs 1,2,3	P<0.005 P<0.001
1800[B]	51.6 ±6.3	38.9 ±2.4	25.4† ±8.5	62.8 ±4.3	1 vs 2,4 3 vs 1,4 2 vs 4	P<0.05 P<0.001 P<0.005
2700[C]	50.3† ±3.5	33.1 ±3.2	§	57.3‡ ±6.4	2 vs 1,4	P<0.005
Significance:	NS	B vs C P<0.05	A vs B P<0.001	A vs B,C P<0.005,0.01		

mean values ± sem, † n=3 ; ‡ n=2 ; § data not available

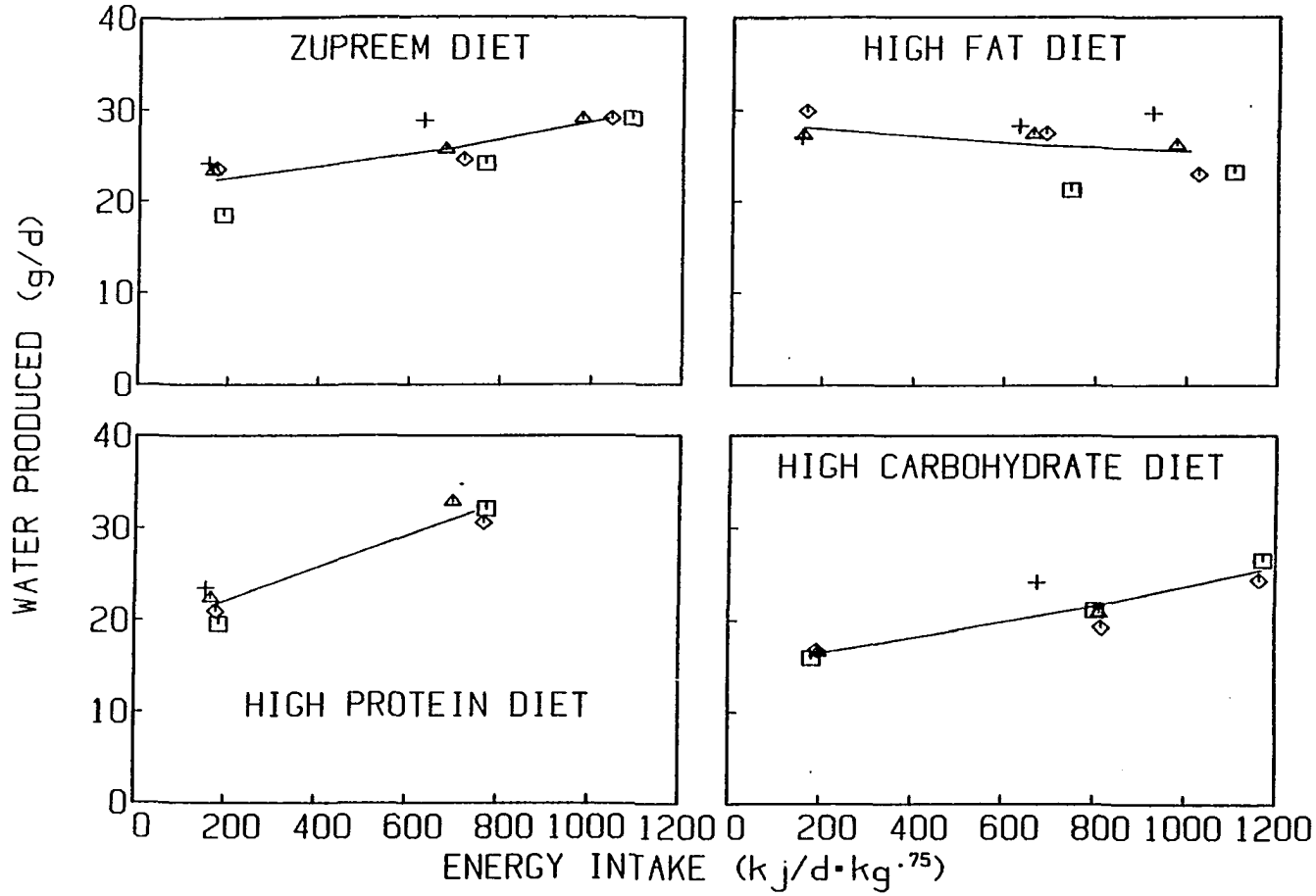

Figure 19. Metabolic water produced by four arctic fox in relation to diet and plane of nutrition.

Table 21. Metabolic water (g/d) of four arctic fox in relation to diet and plane of nutrition. Values calculated from daily urinary nitrogen excretion, oxygen consumption, and carbon dioxide production.

kj/d	ZUP[1]	FAT[2]	PRO[3]	CHO[4]	Significance	
450[A]	22.4 ±1.3	28.1† ±0.9	21.6 ±0.8	16.4† ±0.3	2 vs 1,3,4 4 vs 1,3	P<0.001 P<0.001
1800[B]	25.8 ±1.1	26.0 ±1.6	31.9† ±0.7	21.4 ±1.0	4 vs 1,2,3 3 vs 1,2	P<0.001 P<0.001
2700[C]	29.0† ±0.0	25.5 ±1.5	§	25.4‡ ±1.1	1 vs 2 1 vs 4	P<0.005 P<0.01
Significance:	B vs A,C P<0.005 A vs C P<0.001	A vs B,C P<0.05,0.025	A vs B P<0.001	A vs B,C P<0.001 B vs C P<0.005		

mean values ± sem, † n=3 ; ‡ n=2 ; § data not available

28.1±0.9), CHO (16.4±0.3) the lowest, and ZUP (22.4±1.3) and PRO (21.6±0.8) were intermediate. As the plane of intake increased, the water produced generally increased, especially for the higher protein diet groups, the PRO (MP 31.9±0.7) and ZUP diets. The exception was the FAT group, which experienced only a slightly significant change in metabolic water production.

1.4 Discussion

This study investigated, in the arctic fox, the effect of diet composition and plane of nutrition on the preprandial oxygen consumption, i.e. regulatory DIT, postprandial oxygen consumption, and the difference between them, the obligatory DIT. The results indicate that, depending on the dietary energy content and the ratio of protein, fat, and carbohydrate, DIT can contribute significantly to the total oxygen consumption of the arctic fox.

Zupreem, the base diet used to formulate the other three experimental diets, was highly digestible, with a ADMD of 84%. Studies of the digestibility of canned rations formulated for the feeding of the cat (Felis catus), a carnivorous species that has nutrient requirements similar to those of the arctic fox, indicated lower values than this study, averaging up to 10% lower (NRC, 1978a, 1982; Kendall et al., 1982). Digestibilities of all meat diets in silver fox (Vulpes vulpes) were approximately 10% higher than the values reported here (Inman, 1941; Inman and Smith, 1941).

Zupreem's lower ADMD compared to fresh meat is a reflection of its processing and constituents, e.g. the horsemeat and meat and chicken by-products. Although the

ADMD of Zupreem was lower than that of a fresh meat diet, it was similar to the ADMD observed in carnivore feeding experiments utilizing natural foods (Vogtsberger and Barrett, 1973; Litvaitis and Mautz, 1976; Moors, 1977; Riewe, 1977). Natural foods for fox have a higher proportion of components such as hair, bones, cartilage, tendons, etc. that are hard to digest. The ADMD reported here for Zupreem validates the use of the Atwater values for metabolizable energy content, since the ADMD is very similar to the values used in the calculation of the Atwater constants.

Comparison of the observed rates of oxygen consumption (Tables 5 and 6) to those predicted on the basis of body weight (Kleiber, 1975) depended on the group used for the comparison, i.e. pre- or postprandial, diet type, or plane of nutrition. In the preprandial state the observed metabolic rates of the CHO fed fox were lowest of all the groups (68% LP, 62% MP, and 68% HP of that predicted from body weight). Only the FAT diet group's preprandial metabolic rate exceeded the predicted values (112% LP, 108% MP, and 99% HP). The values for the ZUP and PRO diet groups were intermediate. In the postprandial condition these percentages generally increased, reflecting the DIT associated with each diet.

The greatest increase in percentage was seen in the PRO diet group (83% LP, 117% MP, and 120% HP).

Comparisons of the metabolic rates calculated from oxygen consumption, carbon dioxide production, and nitrogen excretion (Table 14) to the rates predicted by body mass were similarly comparable to the above preprandial relationships, albeit closer.

The rates of postprandial oxygen consumption observed (Table 8) were generally comparable to those observed in Underwood's (1971) metabolic studies of fed arctic fox (0.445 ml $O_2/g \cdot h$ = 10.6 $ml/min \cdot kg^{0.75}$). Underwood's fox were exclusively fed, ad libitum, a high protein diet of caribou flesh, trimmed of all visible fat. Thus, the most valid comparisons with Underwood's study would include the MP diet groups of this study (93%, 98%, 108%, and 81% of Underwood's $\dot{V}O_2$ values for the ZUP, FAT, PRO, and CHO diet groups, respectively), with the PRO diet being most comparable in energy profile (Watt and Merrill. 1963). The LP values were generally lower in comparison, 86%, 104%, 77%, and 66%, while the HP values were higher, 95%, 98%, 111%, and 89%, for ZUP, FAT, PRO, and CHO diets, respectively.

Within each plane of nutrition, the CHO diet was associated with the lowest metabolic rates. Hennemann III et al. (1983) suggest that a high carbohydrate diet may be

an important factor in the low metabolic rates observed in some canids. In their study of the crab-eating fox, Cerdocyon thous, the diet was moderately high in carbohydrate, and the observed metabolic rates were 65%-75% of that expected from body weight. The diet of Cerdocyon thous, similar to Alopex lagopus, varies seasonally, and includes fruits in the wet season. The CHO diet fed to the arctic fox was different from the usual fox diet of microtine rodents, birds and eggs, and carrion, depending on location and season. In natural food, it would be similar to a diet of blueberries and hare, 9 to 1 by weight, and would be available only during the summer, although a high carbohydrate diet might be consumed at any time of the year by those arctic fox feeding at human garbage dumps.

The high fat diet was associated with a regulatory DIT that was significantly greater than that observed in the other diets, which were high in protein or carbohydrate. Fat feeding had no associated obligatory DIT at any plane of nutrition. This contrasts to the considerable obligatory DIT observed in the fox fed the PRO, CHO, and ZUP diets at near and above maintenance energy requirements.

These observations imply that, although FAT obligatory DIT was non-existent, regulatory DIT associated

with fat feeding was very well developed. Conversely, obligatory DIT may well have been present, but was of such a long-term duration, that the periodicity (linked to meal feeding) of the obligatory DIT, as seen in the other three diets, was masked. Explanations for these differences in the fox's responses to the experimental diets lie in the metabolic substrates that would predominate during both the chronic and acute phases of feeding, and their effects on specific tissue sites of thermogenesis.

The interpretation of these results, from an adaptive viewpoint, requires a review of possible biochemical mechanisms. The major mechanism of DIT in several rodent species appears to be centered in BAT (Himms-Hagen 1983). It is not known whether the adult fox has any BAT, but it is a distinct possibility, considering that this tissue has been found in other non-rodents, such as adult humans and young pigs, albeit in smaller concentrations than that found in the neonatal condition (Dauncey et al., 1981; Heaton, 1972; Huttunen et al., 1981; Ricquier et al., 1982; Tanuma et al., 1975). Since BAT activity has been associated with DIT in non-rodent species, including humans and pigs (Lean and James, 1983; Jameson et al., 1983; Rothwell and Stock, 1979), it is reasonable to postulate a role for BAT in the DIT observed in the fox,

as it has been hypothesized in the human (Himms-Hagen, 1984).

The most sensitive and powerful mechanism of thermogenesis in BAT occurs when its mitochondria become uncoupled, i.e. the inner mitochondrial membrane becomes more permeable to the reentry of protons. This proton conductance pathway allows the proton to return to the mitochondria matrix without the concomitant ATP synthesis, according to Mitchell's chemiosmotic coupling theory of oxidative phosphorylation (Lehninger, 1975; Nicholls and Locke, 1983). In such an uncoupled state, the mitochondria oxidize substrate rapidly with energy transfer to heat production rather than coupling to production of high-energy phosphate bonds. Regulation of the proton conductance pathway is thought to involve a protein on the inner mitochondrial membrane. Uncoupling results when inhibition of this regulatory protein is removed.

The activity of the oxidative phosphorylation pathway is directly related to the degree of binding of guanosine 5'-diphosphate (GDP) to the regulatory protein on the inner mitochondrial membrane, a reflection of both the number of regulatory sites and their binding activity. High fat diet has induced, in mice, an increase in BAT GDP binding and mitochondrial respiration, independent of

energy intake, suggesting an increase in the activity of the proton conductance pathway (Mercer and Trayhurn, 1984a). The increase in the proton conductance in the high fat-fed mice may have been associated with dietary lipid inhibition of adipocyte fatty acid synthesis. In theory, this should lead to an increase in resting metabolism of the cell. Similarly, the present study showed a greater level of whole body preprandial respiration, independent of plane of energy intake, compared to the high protein and carbohydrate diets.

A high fat diet, combined with enhanced sympathetic nervous system activity, would stimulate lypolysis and increase the concentration of free fatty acids in the plasma and presumably in mitochondria (McElroy et al., 1986; Schwartz et al., 1983; Young et al., 1982; Levin et al., 1983). These high levels of fatty acids would inhibit lipogenesis, and there would be a concomitant decrease in the requirement for ATP. This would offer more permissive conditions for uncoupling of respiration from ATP synthesis, and consequent increase in oxygen consumption. Also, there is a direct stimulatory effect of the free fatty acids in relieving the inhibition of the regulatory protein on the proton conductance pathway.

Alternatively, when diets high in carbohydrate or protein, but lower in fat content, are fed, lipogenesis

would be stimulated, increasing the requirements for ATP. The increased need for ATP would attenuate the proton conductance pathway and inhibit decoupling of respiration. In the present study, only the high protein, high carbohydrate, and Zupreem diets exhibited lipogenesis (Table 17), whereas the high fat diet was associated with lipid oxidation even at submaintenance intake.

Other mechanisms that result in non-shivering thermogenesis which occur more generally in tissues and organs involve "futile" cycling of substrates most often brought about by local substrate excess, i.e. by mass action phenomena. The substrate cycles would be affected not only by substrate availability, but also by changing hormonal concentrations (insulin, glucagon, catecholamines) influenced by the absorbed nutrients. Relevant substrate cycles could operate in various tissues, such as adipocytes, liver, skeletal muscle, and kidney (Newsholme and Crabtree, 1976; Hue, 1981).

Substrate cycles are important in metabolism because they improve the sensitivity of the flux through a pathway that involves two opposing non-equilibrium reactions. The flux depends on the rates of these two reactions, which cancel one another. Thus, if reaction A proceeds forward at 1000 arbitrary units, and if reaction B catalyzes in the reverse direction at 950 units, then the net flux is

50 units via reaction A. If a specific regulator affects both reaction A and B in a stimulatory and inhibitory manner, respectively, then sensitivity of such a system to regulation is proportional to the rate of cycling (Newsholme and Start, 1973; Newsholme, 1980). The cost for improvement of sensitivity to amplification can be considerable. If the flux is high in such a set of opposing reactions, then there would be a good deal of heat generated, but little actual work performed, hence the title of "futile cycle".

Direct evidence for the generation of heat from cycles has been gathered from investigations of the flight muscles of the bumble bee (Bombus affinis). The "futile" cycle between fructose 6-phosphate and fructose 1,6-diphosphate is thought to contribute to the heat production necessary to raise the flight muscle temperature allowing this heterotherm to forage in cold weather (Clark et al., 1973a). The same cycle was shown to be intimately involved in a malignant hyperthermic condition of pigs (Clark et al., 1973b).

The high levels of circulating free fatty acids from the high fat feeding might also cause increased activity of the associated substrate cycles, (i) the fatty acid synthesis/oxidation cycle, and (ii) the triglyceride/fatty acid cycle, that have been postulated to contribute to DIT

(Newsholme, 1978; Trayhurn, 1981). In the triglyceride/fatty acid cycle heat is produced by repetitive lipolytic and reesterification reactions, and the concomitant hydrolysis of ATP (Newsholme and Crabtree, 1976; Newsholme, 1978). Other substrate cycles that could participate in heat generation are (i) glucose and glucose-6-phosphate, (ii) glycogen and glycogen phosphate, and (iii) phosphoenolpyruvate and pyruvate, although these cycles would be more relevant to the thermogenesis induced by carbohydrate and protein feeding (Katz and Rognstad, 1976).

The increased availability of free fatty acids from the high fat diet may also have caused augmented operation of the glycerophosphate shuttle. This shuttle operates to move reducing equivalents of cytoplasmic NADH into the mitochondria by reduction of dihydroxyphosphate to glycerophosphate. The mitochondrial membrane is permeable to glycerophosphate, not to NADH or dihydroxyacetone. Once in the mitochondria, the glycerophosphate is oxidized to dihydroxyphosphate, with production of mitochondrial NAD. The movement of reducing equivalents in the reverse direction, mitochondria to cytosol, could be effected by the malate-aspartate shuttle. This redox cycle, which could reside predominantly in hepatic tissue, has a potential for heat generation, as no net work is performed

(Berry et al., 1983, 1985). The hepatic location of this metabolic process would obviate the need of BAT to produce heat in the fox. If the reducing equivalents generated by this shuttle are utilized in oxidative phosphorylation, then increased heat production would also result, since the alpha-glycerol phosphate was oxidized by a flavoprotein, producing FADH, which would generate 2 ATP instead of 3 ATP (Stirling and Stock, 1973). If the FAT diet was more slowly absorbed than the other diets, which were lower in fat, then the induction of the above mechanisms would have been maintained over a longer time period. The plasma levels of free fatty acids of dietary origin would have remained relatively constant, compared to the presumed more dramatic diurnal rise and fall of the plasma glucose and amino acid levels that resulted from the rapidly absorbed carbohydrate and protein diets, respectively.

The higher obligatory DIT observed in the PRO, ZUP and CHO diet groups, compared to that in the FAT group, may have been a reflection of the high cost of assimilating the higher levels of protein and carbohydrate in those diets. In the sub-maintenance condition, the synthesis of macromolecules would be minimal, as the nutrients in the diet would be utilized for oxidation to provide for energy needs. At this low plane of nutrition

none of the diet groups exhibited lipogenesis. As the plane of nutrition increased, so did the level of lipogenesis, and the magnitude of obligatory DIT, except for the FAT group, which maintained a constant level of lipolysis. There were highly significant direct correlations between oxidation and intake of protein and carbohydrate with over 80% of the variance accounted for by the regressions

$$PO = 0.00468 + \underset{(0.028)}{0.718\ PI} \qquad r^2=0.947,\ P<0.0001,\ n=38$$

$$CO = 16.3 + \underset{(0.025)}{0.292\ CI} \qquad r^2=0.790,\ P<0.0001,\ n=38$$

where PO and CO represent oxidation of protein and carbohydrate (g/d), and PI and CI represent dietary intake of protein and carbohydrate (g/d).

The historical hypothesis that obligatory DIT is mostly induced by a high protein diet is an attractive one, but is not totally supported by the present experiments. The FAT group was fed a diet low in protein, and exhibited no obligatory DIT; the PRO group was fed high amounts of protein, and manifested the highest obligatory DIT. On the other hand, fox fed the CHO diet exhibited a high obligatory DIT that was as high as that of the PRO and ZUP diets. High protein content was not involved in this case as the CHO diet was low in protein.

Therefore in the arctic fox both protein and carbohydrate are inducers of obligatory DIT. This can be expressed mathematically as the direct and inverse relationships between obligatory DIT and the intake of protein (PI, g/d) and fat (FI, g/d) at MP.

$$\text{MP DIT} = 50.8 + -1.25\ \text{FI} + 0.39\ \text{PI} \qquad r^2=0.791$$
$$(0.29) \quad (0.16) \qquad P<0.0001\ n=15$$

In this study, as fat decreased in the diet, then protein or carbohydrate content increased. This indicates that obligatory DIT depends not on protein content per se, but rather on the metabolic state of degradative and biosynthetic processes induced by the immediate inflow of substrates supplied from the meal. In the fox, carbohydrate is similar to protein in the stimulation of these processes.

High protein intake would stimulate not only higher protein oxidation, but also lipogenesis from protein, which is energetically more costly than from carbohydrate, due to the higher energy cost of deamination and processing, especially for the gluconeogenic amino acids (Flatt 1978; Himms-Hagen 1976). The higher protein intake would also stimulate a higher degree of protein turnover, which has been hypothesized to stimulate DIT (Ashworth, 1969; Ashworth et al., 1973; Coulson and Hernandez, 1979).

No such protein turnover would be stimulated by the high carbohydrate, low protein content of the CHO diet (Klain and Hannon, 1976).

Hormonal balance controls the flow of substrate in the cycling pathways which generate heat. Recent studies show that substrate supply differentially affects hormone levels (Sun et al., 1977; Susini et al., 1979; Landsberg and Young, 1983; Rothwell et al., 1983; Landsberg et al., 1984). Thus, the hormonal profile on the PRO diet might also be different from that manifested on either the FAT or CHO diets. For example, glucagon should be higher on the PRO diet, while insulin would be stimulated by the CHO diet. Insulin has an anti-lipolytic effect and would stimulate lipogenesis and glucose disposal, while glucagon would induce glucose mobilization, via glycogenolysis and gluconeogenesis. For rats with diabetes induced by streptozotocin, cafeteria feeding fails to induce the characteristic increase in oxygen consumption and response to norepinephrine. The response is normalized with insulin replacement, thus suggesting an insulin requirement for DIT (Rothwell and Stock, 1981a).

Diets high in carbohydrates may also produce a catecholamine response which, in acute feeding of glucose in man, is thought to be insulin mediated (Rowe et al., 1979, 1981; Welle et al., 1980, 1981). Similar increases

in norepinephrine turnover have been observed in rats fed sucrose (Young and Landsberg, 1977). The mechanisms induced by these hormonal changes effected by the high carbohydrate and protein diets may have been qualitatively different from those related to the high fat diet.

These metabolic responses in oxygen consumption to diet composition could have adaptive significance to the arctic fox. The absence of obligatory DIT at the low plane of energy intake would allow for conservation of energy resources during winter, when the arctic fox is at great risk of energy deficiency. The increased risk develops not only from the scarcity of food, but also from the energy cost of movements in search of food. The usual prey, microtine rodents, birds, and eggs, of both inland and maritime fox during the summer become scarce during the winter. Most of the birds have migrated south, and the small mammals are protected by snow cover. Although some prey, such as ptarmigan, rodents, hare, and caribou, are still available to those fox that remain inland, many fox move out onto the sea ice in search of food, mostly marine mammal carrion, but also including marine fish and invertebrates, and resident sea birds.

The considerable obligatory DIT observed at the medium and high planes of nutrition would be most advantageous when the fox is below its thermoneutral zone.

This might happen after the spring molt, when the animal, denied the considerable protection of its winter pelage, can be confronted with temperatures in the Arctic of about 0° C. The lack of a linear response of obligatory DIT between the medium and high plane of energy intake may once again reflect a conservation mechanism allowing for acquisition of energy when overeating. This would be most advantageous during the winter, when the food resource is limited. For both those fox on the sea ice and those remaining inland, feeding would be intermittent. When food is located, such as marine mammal carrion for the fox on the sea ice, or carrion for the fox residing inland, it would be maladaptive for the fox to "waste" energy on a scale corresponding to the size of a large meal, as this would preclude the animal from acquiring the optimum percentage of energy from the meal.

If the diet preferences of the fox, during winter on the sea ice, are predominantly for fat, similar to the dietary preferences of the polar bear (Stirling, 1974; Stroganov, 1969), then the fox, consuming such a diet, would be able to maintain a high constant metabolic rate as it travels in its search of carrion. The fact that the polar bear on sea ice prefers fat , does not preclude the arctic fox from the same dietary preference. The fox may

discover carrion independent of the polar bear, or enough fat may remain after the bear has eaten its fill of prey.

A high protein and fat diet also has adaptive significance in relation to the water balance of the arctic fox. During the winter, when the fox only has frozen water available for its needs, the oxidation of fat and protein can provide considerable metabolic water, precluding the necessity of expending energy in melting the available ice. Significant metabolic water can also be produced when the fox is synthesizing fat and oxidizing fat and protein, such as when it feeds on a large amount of carrion, such as a whale or walrus.

The present study shows that DIT contributes significantly to the total oxygen consumption of the arctic fox, and is related to a meal's nutrient composition and total energy content. Obligatory DIT was absent, regardless of the nutrient composition of the meal, when feeding level was below maintenance, but was significantly present for high protein and carbohydrate diets, when fed above maintenance. The level of obligatory DIT did not increase as energy intake increased from near to above maintenance energy requirements. Although a high fat diet failed to produce obligatory DIT, whether fed near, below, or above maintenance energy requirements, it was associated with the highest

regulatory DIT of all the diets tested. These results suggest that DIT may be adaptive as a contribution to thermoregulation in cold stress in the severe conditions of the Arctic.

Chapter 2

Glucose turnover in the arctic fox

2.1 Introduction

Carnivores are similar to ruminants as they must synthesize almost all of their glucose requirements from dietary precursors (Ballard et al., 1969; Newsholme and Start, 1973). Whereas the regulation of gluconeogenesis has been studied extensively in ruminants (see reviews by Ballard et al., 1969, Leng, 1970; Young, 1977), only a few studies have addressed this phenomenon in carnivores, including birds (Migliorini et al., 1973; Veiga et al., 1978) and mammals (Kettelhut et al., 1980). Ruminants are different from carnivores in that their blood (plasma) glucose levels are both lower and less well defended during fasting (see Nelson et al., 1942 and Migliorini et al., 1973 in contrast to Steel and Leng, 1973 and White and Leng, 1980). Blood glucose levels also decline upon fasting in animals consuming high starch diets (e.g. ponies, rats and graniverous birds) (Argenzio and Hintz, 1972; Veiga et al., 1978; Kettelhut et al., 1980).

However, when rats are fed carbohydrate-free diets, either high in protein or fat, they maintain their blood glucose levels upon fasting (Eisenstein et al., 1974; Eisenstein and Strack, 1971; Whitney and Roberts, 1955; and Zaragoza and Felber, 1970). These observations imply that high glucose concentration and its stability upon fasting are due largely to dietary rather than phylogenetic traits.

It has been shown that high blood glucose levels are associated with high rates of glucose metabolism in ruminants (Bergman, 1963; Leng, 1970; Steel and Leng, 1973), whereas Eisenstein et al. (1974) hypothesize that adaptation to carbohydrate-free diets involves a lowered dependency on carbohydrate (glucose) metabolism in both the fed and fasted states, which implies little direct dependency of glucose metabolism on glucose level, or vice versa. This latter hypothesis was subsequently rejected when it was shown that whole body glucose metabolism (total entry rate) was high in the cat, a carnivore, and in the omnivorous rat, when fed low carbohydrate diets (Kettelhut et al., 1980). In general, since glucose total entry rate is a good measure of in vivo gluconeogenesis in animals fed a low carbohydrate diet, the high gluconeogenic capacity and elevated blood glucose levels associated with low carbohydrate diets (Migliorini et al., 1973; Veiga et al., 1978) suggest a mass action effect of

dietary glucose precursors on blood glucose level. The results for ruminants (Leng, 1970, Bergman, 1963) further support this concept. Although mass action effects cannot be used necessarily to account for the defended high glucose levels upon fasting, the phenomenon should support high rates of glucose utilization (measured as glucose irreversible loss and oxidation rates) for an active animal, provided that the precursors are readily available in body tissues.

I could find no discussion in the literature on the evolutionary significance of high glucose levels in carnivores. Since the nervous system is highly dependent on glucose for maintenance and peak performance (Ballard et al., 1969; Cahill et al., 1966; Lindsay, 1970), then a high blood glucose level may convey an advantage through better performance at hunting and/or predator avoidance. Furthermore, because many carnivores experience frequent periods of fasting, a physiologically defended high blood glucose level may again convey an advantage in searching for and capturing carrion or prey. The finding of a high blood glucose level in the subject species of this study, the arctic fox (Penman et al., 1981), is consistent with this hypothesis.

From extensive studies with ruminants it has been shown that the level at which blood glucose is maintained

is determined by the dietary plane of nutrition (Leng, 1970; Judson and Leng, 1968; Lindsay, 1970, McEwan et al., 1976), specifically by the level of intake of dietary glucose precursors. Thus, a high rate of gluconeogenesis may be an essential requirement for high blood glucose levels in the carnivore.

An alternate hypothesis may be that glucose levels reflect the breakdown of dietary precursors for energy metabolism. Hence the flow of dietary carbon through the glucose pool would reflect the requirement for dietary use of protein and fat for energy metabolism (Freeman et al., 1970) and body growth and development (Kempton et al., 1978; Leng and Ball, 1978). The origin of the glucose carbon (i.e. glucogenic amino acids and glyceride-glycerol) would then depend on the protein:fat ratio in the diet and the energy requirements of the animal for maintenance and production. Certainly the rates of glucose metabolism (total entry rate, irreversible loss, and oxidation rate) have been correlated with metabolic rate in ruminants and non-ruminants alike (Ballard et al., 1969; Forichon et al., 1977).

The protein level and protein:fat ratios of diets are also implicated as controlling factors in diet-induced thermogenesis (DIT) (see Chapter 1). Thus, a role for glucose metabolism in this process can be inferred.

However, whether glucose metabolism is metabolically involved in DIT is not known. A suggested mechanism is outlined in Chapter 1, in which the generation of heat takes place via the futile cycling of glucose and glucose-6-phosphate. This occurs without loss of glucose carbon and the energy source is high energy phosphate bonds generated from ATP (Katz and Rognstad, 1978).

In the arctic fox, DIT is high (35% increase in metabolic rate) in the maintenance-fed animal offered a low carbohydrate diet of mixed protein and fat (Table 9, Chapter 1). If futile cycling of glucose plays a role in this process, then it would be expected that glucose resynthesis, the difference between total entry rate, estimated with [2-^{3}H] glucose, and irreversible loss, estimated with [U-^{14}C] glucose (Katz et al., 1976) would be high for diets that elicit a high DIT. In this study, glucose resynthesis in the maintenance fed fox is measured and compared with other species to test this hypothesis.

During fasting glucose total entry rate is proportional to fasting metabolic rate in sheep (White and Leng, 1980). The amount of glucose formed de novo is set by the rate of body reserve mobilization (Leng, 1970), of which 37% is derived from amino acids (Linsay, 1979). Glucose recycling processes, which conserve glucose carbon, are used to raise the total rate of glucose

synthesis to some minimal requirement that is apparently proportional to fasting metabolism (White and Leng, 1980). Whether this relationship is demonstrable in fasting carnivores is not known. In chapter 1, the relationship of metabolic rate, measured 18 h after feeding, to diet type and intake shows that the previous level of intake influences metabolism in the arctic fox fed a mixed protein and fat diet, or one high in either carbohydrate or protein. If the findings for sheep can be extrapolated to the arctic fox, then glucose total entry rate should parallel the metabolic rate findings shown in Figure 6, Chapter 1.

A limited test of hypotheses surrounding the defense of blood glucose level by gluconeogenesis and the association of DIT with glucose recycling and overall metabolism was performed by comparing the fed and fasted glucose total entry rates of arctic fox fed, at maintenance, a low carbohydrate mixed protein and fat diet, similar to the Zupreem diet used in Chapter 1.

2.2 Materials and Methods

2.2.1 Animals

The four arctic fox used in this study were two years old. They were fed a commercially canned diet, Friskies Poultry Platter (Carnation, Los Angeles, CA) once daily, at a level to maintain a mean weight of 4 kg, i.e approximately 2000 kj/d metabolizable energy in the form of 34% protein, 55% fat, and 11% carbohydrate (based on manufacturer's analysis). This closely approximates the analysis of Zupreem (Table 1, Chapter 1). The fox were maintained on this diet for several months with free access to water, and were kept in separate outdoor pens equipped with shelter boxes. Trials were made in the months of March and April when the typical mean ambient temperatures were between -13° and -1° C.

2.2.2 Experimental procedure

On the morning before an experimental trial with the fed fox, a catheter was aseptically inserted into a jugular vein while the fox was lightly sedated with Rompun (xylazine, Haver-Lockhart, Cutter Laboratories, Shawnee, Kansas). The fox was then returned to its outdoor pen to spend the night. On the following morning, the fox was brought into the laboratory and a single injection of 55 μCi of D-[U-^{14}C] glucose (Radiochemical Centre, Amersham,

England) and 85 µCi of D-[2-^{3}H] glucose (New England Nuclear, Boston, Massachusetts), in a total volume of 1.5 ml sterile saline, was injected via the indwelling jugular catheter. The catheter was immediately flushed with two 5 ml volumes of sterile saline. Blood samples (1.0 ml) were then drawn into heparinized tubes at approximately 2, 4, 6, 8, 10, 15, 20, 30, 45, 60, 90, 120, 150, and 180 minutes after the initial injection. Samples were immediately centrifuged, and the plasma and erythrocytes separated. Plasma was stored at -30° C until analyzed, while the erythrocytes were resuspended in saline and kept on ice until the conclusion of the trial, when they were reinjected into the fox. If the trial was with a fed fox, the fox was returned to its outdoor pen, with its jugular catheter still patent, ready for the subsequent fasted experiment the following day. At the conclusion of the fasted experiment, the catheter was removed under Rompun sedation.

2.2.3 Glucose measurements

Plasma glucose concentration was assayed according to the glucose oxidase method, utilizing ABTS as the chromogen (Bergmeyer and Bernt, 1974). Glucose was isolated as the penta-acetate derivative (Jones, 1965), dissolved in a commercial cocktail (Spectra-fluor,

Amersham, Arlington Heights, IL), and radioassayed with a Beckman scintillation system (LS-7500).

2.2.4 Calculations

After the initial dose of radioactive glucose, the decrease in specific radioactivity of plasma glucose over time is described by the sum of all exponential terms in the equation:

$$SR_t = \Sigma A_i e^{-m_i t}$$

where t is the time after the injection, SR_t is the plasma glucose specific radioactivity (dpm per mg glucose) at time t, A_i is the zero-time intercept (dpm per mg glucose) of each exponential component, m_i is the rate constant (min^{-1}) of each component, i is the number of components (White et al., 1969).

The natural logarithm of specific radioactivity of plasma glucose versus time was graphed and preliminary estimates of A_i and m_i were made by a "peel-off" process (Gurpide, 1975). These estimates served as initial values for the computer program, SAAM 27, which fitted the final parameters to the data (Berman and Weiss, 1978; Berman et al., 1982). From these data the following variables of glucose metabolism were calculated (White et al., 1969).

Glucose pool size, Q (g), is defined as the amount of glucose with which the injected dose mixes in the fox, i.e. the sampled compartment. It is equal to the ratio of

dose (P, dpm) to the specific radioactivity (dpm/g) at zero time:

$$Q = \frac{P}{\Sigma A_i}$$

Glucose space is defined as the volume with which the pool equilibrated, and is expressed as a percentage of body weight (kg):

$$\% \text{ SPACE} = \frac{Q \cdot 100}{[GLU] \cdot BW}$$

where [GLU] is the concentration of plasma glucose (g/l).

Total entry rate (TER, mg/min) represents the rate of entry of all glucose into the sampled compartment, and is estimated using the 2-^{3}H-glucose dilution curve according to the equation:

$$TER = \frac{Q}{\Sigma\{A'_i \cdot m_i^{-1}\}}$$

where A'_i = fractional zero time intercept, i.e. - $A'_i = A_i/\Sigma A_i$ (Judson and Leng, 1972). Irreversible loss (IL, mg/min) represents the rate of loss from the pool of glucose carbon that will not return to the pool. This estimate was made using the U-^{14}C glucose data according to the equation:

$$IL = \frac{Q}{\Sigma\{A'_i \cdot m_i^{-1}\}}$$

Each estimate represents the dose divided by the area under each respective specific radioactivity curve from time zero to time infinity (White et al., 1969; Judson and Leng, 1972).

Glucose recycling (R, mg/min) is equal to the difference between total entry rate and irreversible loss (i.e. R = TER - IL). It can also be expressed as a percentage of the total entry rate. Glucose turnover time (TT, min) represents the time required to replace the glucose pool by all sources of glucose. It is calculated as the pool size divided by the total entry rate (i.e. TT = Q/TER).

Statistical analysis was performed using the paired Student's t test (BMDP, 1985).

2.3 Results

Semi-log plots of plasma glucose radioactivity (dpm/mg of glucose) versus time (min) curves following the single injection of 2-^{3}H glucose, in typical trials comparing a fed with a fasted fox, are presented in Figure 20. The solid lines represent the calculated best fit to the data using SAAM 27 (Berman, 1978). The summarized exponential analysis of all trials is shown in Table 22 and the fit for each trial is given as the residual variance as recommended by White et al. (1969). Variance about the line tended to be higher for the fed than the fasted fox for both isotopes. Both 2-^{3}H and U-^{14}C glucose were cleared from the plasma more rapidly in the fed state than in the overnight fasted condition.

Body weights of the fox decreased significantly ($P<0.025$, 3.96 to 3.72 kg) after fasting (Table 23). This change represented the daily change in body weight associated with once per day feeding.

Plasma glucose concentrations were fairly constant throughout each trial; the coefficient of variation ranged from 5% to 14% (14 samples) and a significant decline ($P<0.05$) in concentration over the trial period was noted in only three trials (i.e. fox V, Z, J, all when fasted). A paired t test showed that there was no significant

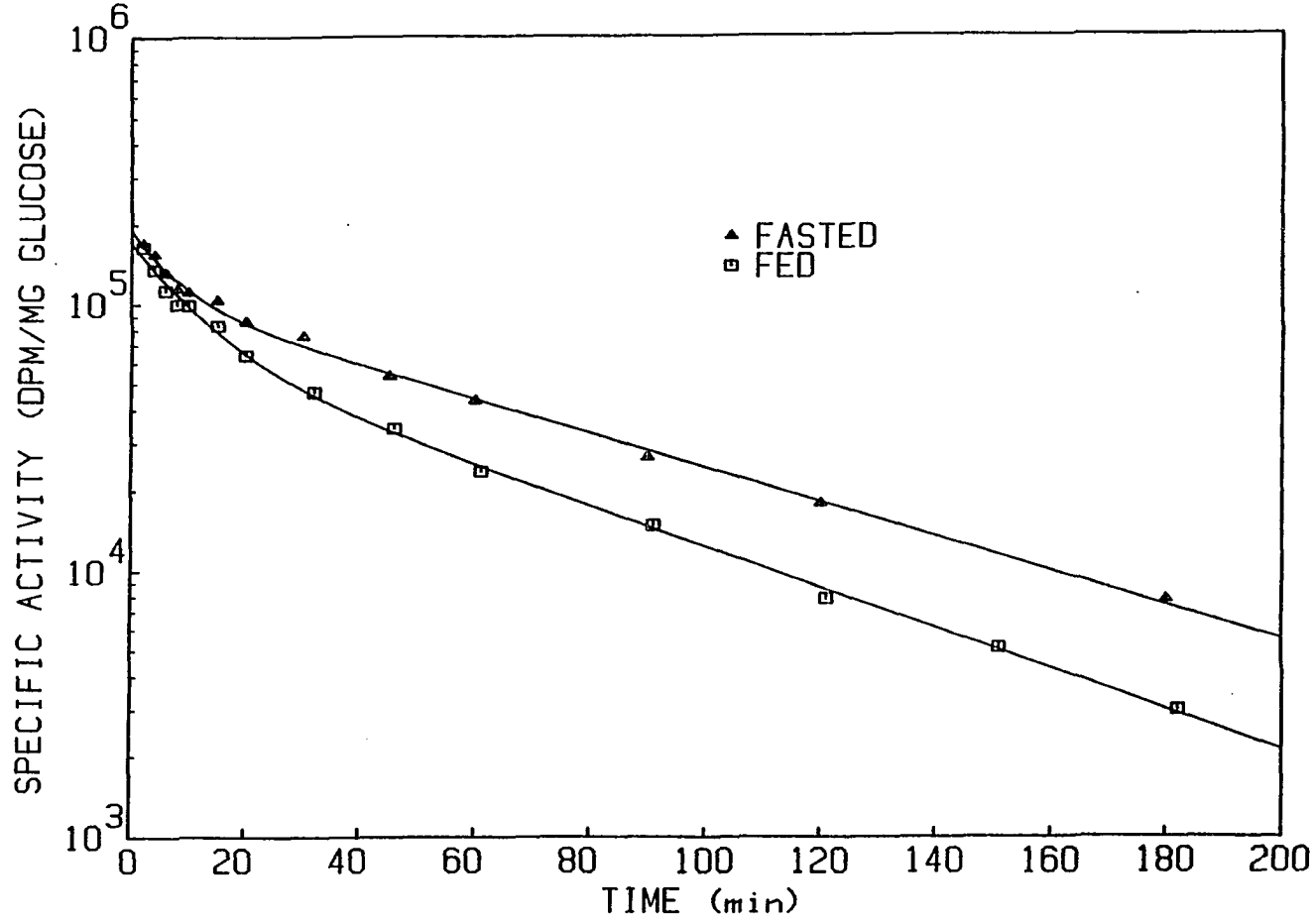

Figure 20. Semilogarithmic plot of plasma glucose specific activity versus time in a fed and fasted arctic fox.

Table 22. Equations[a] of best fit for calculation of glucose kinetics in fed and fasted arctic fox estimated with single injections of 2-^{3}H and U-^{14}C glucose.

Fox trial		Intercepts($\cdot 10^{3}$)[b]		Exponents($\cdot 10^{-3}$)[c]		Residual Variance[d]
		A_1	A_2	m_1	m_2	
V Fed	^{3}H	135.	121.	127.	19.2	0.0214
	^{14}C	78.6	67.7	103.	14.7	0.0257
Fasted	^{3}H	128.	395.	395.	13.9	0.0269
	^{14}C	66.5	89.0	314.	10.7	0.0245
Z Fed	^{3}H	99.6	70.2	94.4	17.5	0.0327
	^{14}C	57.1	43.8	89.3	13.7	0.0258
Fasted	^{3}H	83.5	106.	138.	14.8	0.0267
	^{14}C	45.9	69.8	150.	9.18	0.0217
K Fed	^{3}H	119.	114.	223.	21.4	0.0597
	^{14}C	58.0	60.5	108.	16.3	0.0843
Fasted	^{3}H	127.	129.	214.	12.1	0.0076
	^{14}C	67.0	79.3	191.	9.41	0.0111
J Fed	^{3}H	62.0	149.	195.	32.6	0.0626
	^{14}C	53.2	62.0	70.1	23.2	0.0650
Fasted	^{3}H	110.	165.	172.	15.0	0.0165
	^{14}C	70.5	105.	176.	11.7	0.0157

[a] Specific radioactivity = $A_1 \cdot e^{-m_1 t} + A_2 \cdot e^{-m_2 t}$, where t is the time after injection of 2-^{3}H and U-^{14}C glucose.

[b] Intercept units are dpm/(mg glucose).

[c] Exponent units are min^{-1}.

[d] Residual variance is defined as $\sum\left(\frac{\text{observed value-expected value}}{\text{expected value}}\right)^2$ and is included to help in comparing the goodness of fit of the observed results between different experiments.

Table 23. Glucose metabolism in fed and fasted arctic fox, estimated with single injections of 2-^{3}H and U-^{14}C glucose.

	Fed	Fasted	
Body weight (kg)	3.96 ± 0.11	3.72 ± 0.10	b
Plasma glucose (mg/dl)	134 ± 8	133 ± 10	n
Glucose pool (mg)	944 ± 77	796 ± 73	b
Glucose space (%)	17.9 ± 0.8	16.1 ± 0.5	a
Total entry rate (mg/min·kg$^{0.75}$)	12.0 ± 1.1	7.0 ± 0.6	a
Irreversible Loss (mg/min·kg$^{0.75}$)	10.3 ± 1.0	5.1 ± 0.1	a
Glucose recycling (mg/min·kg$^{0.75}$)	1.7 ± 0.2	1.8 ± 0.5	n
Glucose recycling (%)	14.2 ± 1.2	25.3 ± 4.4	b
Turnover time (min)	28 ± 1	43 ± 1	a

Values are mean ± SEM for 4 fox. Superscripts indicate level of significance: a, $P<0.01$; b, $P<0.05$; n, NS $P>0.05$.

difference ($P>0.05$) between the mean plasma glucose concentration of the fed and fasted fox (Table 23).

Parameters of glucose kinetics are presented in Table 23, and were calculated, as outlined in the methods section, from the individual equations given in Table 22. The mean glucose pool size of the fed fox was significantly greater ($P<0.05$) than that of the fasted fox and the reduction in pool size during fasting was associated with a small but significant reduction in glucose space ($P<0.01$). Glucose total entry rate and irreversible loss were significantly greater ($P<0.01$) in fed fox than during fasting. However, the absolute amount of glucose recycled, 1.7 to 1.8 $mg/min \cdot kg^{0.75}$, was not significantly different ($P>0.05$) between the fed and fasted states. This amount of glucose represented 14% of TER in the fed fox, and 25% in the fasted animals. Turnover time of the glucose pool increased significantly ($P<0.005$) by nearly 50% when the fox were fasted.

2.4 Discussion

The high plasma glucose concentrations reported here suggest that the arctic fox has a well developed mechanism for maintaining high glucose levels (134 mg/dl) on this typical carnivore diet, which is low in carbohydrate and high in protein and fat. These glucose levels are comparable to those determined in previous studies of wild captive and free-ranging mammalian carnivores. Penman et al. (1981) have previously reported values of 178 mg/dl in arctic fox, while others have noted concentration ranges of 115-143 mg/dl in big cats, such as lions (*Panthera leo*) and tigers (*Panthera tigris*)(Fowler, 1986).

High plasma glucose has also been found in some wild and semi-domesticated ruminants, which, like carnivores, must depend on gluconeogenesis for maintenance of plasma glucose, since little glucose is absorbed from their alimentary tracts. For example, the plasma glucose level of caribou and reindeer (*Rangifer tarandus L.*), at 109 to 119 mg/dl (White and Luick, 1976), is considerably higher than for domestic sheep and cattle (43-83 mg/dl) (Bergman et al., 1974; Kronfeld, 1977; Leng, 1970; Schmidt and Keith, 1983). Chandrasena et al. (1979) measured blood glucose concentrations greater than 125 mg/dl in the camel (*Camelus dromedarius*). Although the high plasma glucose

values in wild animals could reflect stress resulting from the sampling techniques, this is not a satisfactory explanation as reindeer can be extremely well trained. The arctic fox in the present study appeared calm during the trials, usually lying in a resting posture in their holding cage while their blood was sampled. This suggests that high plasma glucose concentrations may be of adaptive significance for wild animals in general. A test of the hypothesis that high glucose levels were attributable to diet rather than phylogenic factors is provided by studies with non-carnivorous laboratory animals that have their gluconeogenic mechanisms induced by feeding low carbohydrate diets, similar to the one used in this study. These animals typically exhibit plasma glucose concentrations lower than the values reported for the arctic fox (Table 22) and other wild species. For example, Belo et al. (1976b) observed plasma glucose levels of 102-111 mg/dl in the dog, while Kettulhut et al. (1980) reported values of 80 mg/dl in the cat, and 85 mg/dl in the rat. The hypothesis is therefore rejected.

These high glucose levels in wild species, both carnivorous and ruminant, suggest they may be highly adaptive under wild conditions. Since survival of wild animals depends on optimum processing of sensory input by the central nervous system, a high central demand on

glucose would be expected, as glucose is the sole energy source of nervous tissue. Both the taking of prey, in the case of the carnivore, and avoidance of predators, in the case of the ruminant, are processes that assert maximum selective pressure on the individual. Thus, an adaptation to maintain high plasma glucose levels would be of selective advantage.

Some indication of the selective advantage is given by trends during fasting. The plasma glucose level of arctic fox is resistant to short term fasting. This phenomenon has also been observed in other carnivorous species. For instance, the black vulture (Coragyps atratus) maintains its high plasma glucose concentration (170 mg/dl) through several days of fasting (Migliorini et al., 1973), as does the kelp bass (Bever et al., 1977), the horned owl (Nelson et al., 1942), and the cat (Kettelhut et al., 1980). For the cat, defense of blood glucose is lessened if the diet is high in carbohydrate. For the domestic dog, which has been subject to much genetic change due to selective breeding, plasma glucose concentration shows no resistance to fasting, whether the diet is devoid of carbohydrate or not (Belo et al., 1976b). However, high carbohydrate diets do produce high plasma glucose levels in the fed animal.

Glucose as a metabolic substrate not only influences target organs in relation to concentration, but also by mass action effects, as measured by the glucose pool size (White and Leng, 1980). For the fed and fasted fox in this study, pool sizes (fed, 336 mg/kg$^{0.75}$; fasted, 297 mg/kg$^{0.75}$) are comparable to values reported for dogs fed similar carbohydrate-free diets (fed, 344 mg/kg$^{0.75}$; fasted, 313 mg/kg$^{0.75}$) (Belo et al., 1976b). The differences between fed and fasted animals are significant ($P<0.05$). The size of the carrier pool in which the glucose pool is distributed, i.e. glucose space, is also comparable to that of the carbohydrate starved dog (Belo et al., 1976b). In the arctic fox, glucose space decreased slightly ($P<0.01$) during fasting, suggesting that the size of the extra-cellular fluid pool, in which glucose is distributed, also decreased with fasting. This contrasts with the dog, pony, and sheep, in which the space was maintained on fasting (Table 24). Insulin is known to effect glucose space (Kronfeld and Raggi, 1964), hence the decrease in glucose space in the arctic fox may indicate a diminished role for insulin upon fasting.

Although some of the definitive work on the role of gluconeogenesis in defending a high blood glucose concentration has been done in carnivorous birds, e.g. owl and vulture (Nelson, 1942; Migliorini et al., 1973), these

authors have not estimated whole body glucose turnover. A summary of other interspecies estimates with which to compare the arctic fox is shown in Table 24. Care should be taken in making comparisons as TER and IL in the fed state have been shown to increase with food intake in sheep, reindeer and dogs (Judson and Leng, 1968; Leng, 1970; Lindsay, 1970, McEwan et al., 1976; P. G. Tallas and R. G. White, unpublished observations of dogs). As a broad generalization, TER in only the dog and rat fed high carbohydrate diets are equal to or exceed the arctic fox. All those fed low carbohydrate diets are lower (Table 24). This dietary effect is carried through the fasting state, i.e. a significant decline was common to all species. The same trends may be common to IL too, however there are too few estimations and the only species with an IL as high as the arctic fox was the rat fed a high carbohydrate diet (Katz et al., 1976, Table 24). Estimations for the pony, reindeer, and sheep were 50% of the arctic fox's IL, or less. The IL of the fed and fasted camel (Chandrasena et al., 1979) and the fasted cynomolgus monkey (Macaca fascicularis) (Armstrong et al., 1979) were closest to the arctic fox.

Table 24. Species comparisons of various parameters of glucose metabolism.[a]

Species	%S	TER	IL	R	%R	D[b]	T[c]	Ref.[d]
fox fed	18	12.0	10.3	1.7	14	LC	{1,5}	(1)
fasted	16	7.0	5.1	1.8	25	LC	{1,5}	(1)
cat fed	-	7.1	-	-	-	HC	{1}	(2)
cat fasted	-	3.5	-	-	-	HC	{1}	(2)
cat fed	-	7.2	-	-	-	HP	{1}	(2)
cat fasted	-	4.2	-	-	-	HP	{1}	(2)
dog fed	23	17.8	-	-	-	LPHC	{1}	(3)
fasted	23	9.8	-	-	-	LPHC	{1}	(3)
dog fed	17	10.3	-	-	-	HPHC	{1}	(3)
fasted	16	7.3	-	-	-	HPHC	{1}	(3)
dog fed	20	9.8	-	-	-	LPLC	{1}	(3)
fasted	20	8.4	-	-	-	LPLC	{1}	(3)
dog fed	16	7.3	-	-	-	HPLC	{1}	(3)
fasted	18	6.9	-	-	-	HPLC	{1}	(3)
monkey fasted	-	6.8	4.2	2.6	38	HC	{1,5}	(4)
rat fed	-	6.2	-	-	-	HC	{1}	(2)
rat fasted	-	3.1	-	-	-	HC	{1}	(2)
rat fed	-	12.3	10.0	2.3	19	HC	{1,7}	(5)
rat fasted	-	10.2	7.2	3.0	29	HC	{1,7}	(5)
rat fasted	-	7.5	4.8	2.7	36	HC	{1,5,6,7}	(6)
rabbit fasted	-	5.7	4.0	1.7	30	HC	{1,5}	(7)
pig fasted	-	7.8	5.7	2.1	27	HC	{1,5}	(8)
pony fed	21	-	3.0	-	-	HC	{5}	(9)
pony fasted	21	-	5.9	-	-	HC	{5}	(9)
pony fed	9	6.5	5.0	1.5	23	HC	{1,5}	(10)
pony fasted	9	3.5	2.8	0.7	20	HC	{3,5}	(10)
pony fasted	9	4.3	3.6	0.7	16	HC	{2,5}	(10)
reindeer fed	36	6.7	4.3	2.4	36	HC	{2,6}	(11)
camel fed	-	-	7.0	-	-	HC	{4}	(12)
camel fasted	-	-	4.2	-	-	HC	{4}	(12)
sheep fed	-	-	4.3	-	-	HC	{4}	(12)
sheep fasted	-	-	2.6	-	-	HC	{4}	(12)
sheep fed	21	7.3	6.2	1.1	15	HC	{1,5}	(13)
sheep fed	-	3.8	3.6	0.2	5	HC	{6,7}	(14)
sheep fed pg	-	4.4	3.8	0.6	14	HC	{6,7}	(15)
sheep fed	20	4.2	4.0	0.2	5	HC	{7,8}	(16)
sheep fed	15	6.8	4.6	2.2	32	HC	{5}	(17)
sheep fed	16	4.8	3.9	0.9	19	HC	{5}	(18)
sheep fasted	13	3.7	1.8	1.9	51	HC	{5}	(18)

[a] Abbreviations are as follows: S = space, R = recycling, %R = %recycling, pg = pregnant. Units are $mg/min \cdot kg^{0.75}$.

[b] Diets: L low ; H high; C carboydrate; P protein.

[c] Techniques with various isotopes of glucose: (1) single injection (SI) of 2-^{3}H; (2) SI of 3-^{3}H; (3) SI of 6-^{3}H; (4) SI of 6-^{14}C; (5) SI of U-^{14}C; (6) primed infusion (PI) of 2-^{3}H; (7) PI of U-^{14}C; (8) PI of 6-^{3}H.

[d] References: (1) present study; (2) Kettelhut et al., 1980; (3) Belo et al., 1976b; (4) Armstrong et al., 1979; (5) Katz et al., 1976; (6) Katz et al., 1974; (7) Dunn et al., 1976; (8) Trayhurn et al., 1981; (9) Argenzio et al., 1972; (10) Anwer et al., 1976; (11) Luick et al., 1973; (12) Chandrasena et al., 1979; (13) Leonard et al., 1977; (14) Emmanuel and Edjtehadi, 1981; (15) Hodgson et al., 1980; (16) Judson and Leng, 1972; (17) White et al., 1969; (18) White and Leng, 1980.

Thus, although the arctic fox defends a constant blood glucose level on fasting, glucose TER and IL decline to an extent similar to those species with a less well defended glucose level. This strongly suggests that factors regulating glucose level and turnover are controlled or regulated somewhat differentially in the arctic fox. It could be argued that the blood glucose level is maintained during fasting by decreasing the pool size and space. The contraction of glucose space, from 18% to 16% body weight, may deny some organ component, tissue, or cell compartment a ready glucose supply, which would spare glucose for those with a high requirement. The results and the interpretation support the hypothesis that high blood glucose levels in this carnivore, associated with high whole body glucose turnover, are attributable to phylogenic factors, but a wider species comparison is required to substantiate this differential effect in wild carnivores.

With respect to its glucose metabolism, the arctic fox behaves as if it is absorbing considerable amounts of glucose. Its gluconeogenic capacity is stimulated to such a degree that the metabolic production of glucose approaches inputs of glucose absorbed in animals consuming a carbohydrate-rich diet. Based on the estimated

proximate analysis of the diet, the potential generation of glucose from the protein, fat, and carbohydrate would be 9.7 mg/min·kg$^{0.75}$, which compares favorably with the synthesis that must balance IL, 10.3±1.0 mg/min·kg$^{0.75}$ (Table 23). Of the glucose synthesized from the diet, approximately 33% was derived from dietary carbohydrate, 59% from protein, and only 8% from glyceride-glycerol. This calculation shows the high glucogenic potential from protein and the findings confirm results based on protein excretion (Table 20, Chapter 1). If all this glucose derived from dietary carbohydrate was oxidized, it would produce 78 kj/d·kg$^{0.75}$, which compares favorably with the energy expenditure from carbohydrate oxidation, estimated from respiratory and urinary analysis of a arctic fox fed a similar diet (Table 14, Chapter 1).

The test of hypothesis that glucose recycling is high in animals with high DIT and may be part of the mechanism, can be made by comparing 2-^{3}H with U-^{14}C metabolism. Lower specific radioactivities in 2-^{3}H glucose compared to U-^{14}C are attributable to the recycling of glucose through molecules such as lactate and glycogen, where the 2-^{3}H label would be lost but not the U-^{14}C (Katz and Dunn, 1967). However, the use of 2-^{3}H glucose may have overestimated TER as futile cycling between glucose and glucose-6-phosphate would cause a loss of the label with

no net synthesis or loss of glucose via metabolic pathways (Katz and Rognstad, 1978). Although there are no data available assessing the differences between the various tritiated glucoses in small mammalian carnivores, experiments with rats suggest that the differences between total entry rates determined with 2-^{3}H glucose and 6-^{3}H glucose are in the range of 10-20% (Katz et al., 1976).

Percent recycling in fed fox was greater than values seen in several studies of fed sheep. Judson and Leng (1972) calculated 5% using 2-^{3}H and U-^{14}C glucose, as did Emmanuel and Edjtehadi (1981). Luick et al. (1973), however, in the fed semi-wild reindeer, measured percent recycling to be 36%. The fed fox's values for percent recycling were most closely approximated by Leonard et al.'s (1977) 15% in fed sheep and by Katz et al.'s (1976) 19% in fed rats. Fasted fox exhibited a recycling percentage (25%) that was generally lower than that seen in several other fasted species: 30% in the rabbit (Dunn et al., 1976; Katz et al., 1974), 32% in the monkey (Armstrong et al., 1979), 36% in the rat (Katz et al., 1974), 31% in the dog (Belo et al., 1976a), 27% in the pig (Trayhurn et al., 1981), and 51% in the sheep (White and Leng, 1980) (Table 24.). The fox's lower absolute rate of recycling during fasting, compared to several other species, including the monkey, rat, reindeer, and sheep

(Table 24) suggests that its gluconeogenic mechanisms can produce glucose more effectively from glycerol in stored fat, or from protein reserves. However, since the absolute rate of recycling did not differ significantly from the fed to fasted condition in the arctic fox (Table 23), then the hypothesis that the heat generated during glucose recycling contributes to DIT must be rejected.

The main purpose of this experiment was to assess glucose metabolism in both the fed and fasted arctic fox maintained on a low carbohydrate diet, similar to the diet it would find in its natural habitat. It was determined that the capacity for glucose production in this arctic carnivore is highly developed. When the arctic fox feeds on a carbohydrate-free diet, its glucose utilization is similar to that of animals consuming high carbohydrate diets.

Conclusions

This study is one of the first to address the metabolism of substrate utilization in a wild mammalian carnivore. Carnivores, like ruminants, are unique in their dependency on gluconeogenesis, to supply energy to several body tissues, such as the brain. The objectives of the foregoing study of the arctic fox were, first, to determine the diet induced thermogenesis associated with four diets that varied in the proportion if the major nutrients, fat, protein, and carbohydrate; second, to characterize the association of those individual nutrients with DIT; and third, to assess glucose utilization in fed and fasted arctic fox, maintained on a diet low in carbohydrate and high in protein and fat, similar to its natural diet.

The results of testing the hypotheses proposed in the foregoing chapters indicate the following:

1) DIT contributes significantly to the total heat production of the arctic fox.

2) DIT can be considered adaptive as it represents a significant increase in the metabolic rate of an arctic

fox fed, at maintenance level, a diet similar in composition to its natural diet (high in protein and fat) and thus may contribute to thermoregulation during cold stress.

3) DIT, as a measure of the increase in metabolic rate from the fasting to fed condition, is related to the energy level of the diet. At low levels of energy intake, when weight loss is apparent, DIT is non-existent, and thus is adaptive as it conserves body energy stores. Conversely, DIT is attenuated when the fox feeds on large meals. Such a situation is most probable when the arctic fox locates large carrion during the winter. Gorging on the food would cause the fox to begin to gain weight, as opposed to the weight loss apparent with the intermittent feeding associated with the usual food scarcity of winter. This aspect of DIT can be considered adaptive as it would assure the arctic fox of the optimum percentage of a meal's energy content.

4) When the arctic fox is fed a low carbohydrate diet high in protein and fat, then, in the fed state, glucose metabolism, i.e. total entry rate and irreversible loss, is high compared to other animals, similarly reliant on gluconeogenesis to supply energy to glucose dependent tissues. The high level of glucose metabolism may support the high blood glucose concentrations of the arctic fox

and its resistance to the effects of fasting. This supports the contention that a well defended high blood glucose level is a phylogenic trait of carnivores, which must maintain their nervous systems at peak performance despite intermittent periods of fasting. However, since glucose total entry rate and irreversible loss decrease during fasting, other factors must be involved in the defense of blood glucose concentrations during fasting, such as alterations in glucose space.

5) The absolute amount of glucose recycling was not significantly different between the fed and fasted fox, fed a low carbohydrate diet. This result suggests that glucose recycling is not an important determinant of DIT.

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Appendix A. Body weights (kg) of four arctic fox on eighth day of feeding trial (Chapter 1)

Diet	kj/d	Fox id [a] V	Z	J	T
ZUP	450	3.04	4.02	3.39	3.65
	1800	3.26	4.21	3.44	3.70
	2700	3.56	4.42	3.69	4.01
FAT	450	3.27	4.00	3.65	3.86
	1800	3.23	4.13	3.62	3.80
	2700	3.41	4.35	3.70	3.99
PRO	450	3.00	3.85	3.13	3.49
	1800	3.18	4.04	3.13	3.60
	2700	3.15	----	3.34	----
CHO	450	2.91	3.69	2.88	2.83
	1800	2.99	3.72	2.87	2.98
	2700	3.21	3.91	3.21	3.19

[a] Corresponding symbols used in the figures of Chapter 1 are the square, cross, diamond, and triangle, respectively.